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International management strategies for trace organic compounds in waterbodies and supporting advanced analytical techniques

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Abstract

The occurrence of trace organic compounds (TOrCs) in waterbodies is a worldwide threat for environmental water quality. Although detectable concentrations of TOrCs are comparably low, there is a great concern about adverse health effects. As a response, many countries have started to implement strategies for the management of TOrCs. These strategies are often adjusted to site-specific differences, addressing different occurrence patterns of TOrCs, but also to accommodate environmental, geographic and economic conditions. Thus, proposed or implemented management strategies favored in different countries seem to be hardly comparable. Some strategies focus on the control of a few toxicologically characterized compounds, while others aim to minimize TOrCs discharge from selected sources using end-of-pipe approaches. Independent from the focus of the individual management strategies, appropriate analytical techniques play a major role for the determination of water quality and the identification of TOrCs in the aquatic environment.

The aim of this study was to investigate the underlying principles of proposed and implemented management strategies for TOrCs worldwide, to identify the analytical requirements of the strategies, and to evaluate how advanced analytical techniques can contribute to the establishment of more holistic management strategies for a more comprehensive protection of environmental and human health.

In a first step, strategies for the management of TOrCs from the United States of America (U.S.), Australia, the European Union (EU) and Switzerland were evaluated. The strategies were investigated to understand their motivations and underlying paradigms. It could be shown that strategies consist of toxicity- and/or emission avoidance-based principles. The strategies implemented in the U.S., Australia and the EU contained toxicity-based principles to different extents. The Swiss and EU strategies were (partly) based on the emission avoidance based principle. Toxicity based principles are well-suitable for the management of a limited number of well-known and characterized compounds, but can hardly be applied to control unexpected risks, posed by unassessed or unknown compounds and compound mixtures. Emission avoidance based principles, can minimize unexpected risks, but an implementation for all sources of TOrCs is hardly possible. It was concluded that a combination of both principles might have the highest impact to minimize the occurrence of TOrCs in waterbodies. Toxicity based principles can be established to control known, hazardous compounds and emission avoidance principles can limit the discharge of TOrCs and minimize unexpected risks.

In order to monitor the occurrence of TOrCs, verify compliance with existing water quality standards, and to identify unknown compounds, powerful analytical tools are needed for all management strategies. A comparison of the individual analytical requirements revealed that all strategies use the

same set of analytical strategies in different extents, namely target-, suspects, and non-target screening. Analytical techniques commonly rely on reversed phase liquid chromatography (RPLC) with mass spectrometric detection. This technique is well-established and suitable for the separation and detection of medium to non-polar compounds. With increasing knowledge about the origin and fate of TOrCs, it became obvious that the chemical spectrum of TOrCs is broader than the range of compounds separable by RPLC. Especially for the separation of very polar compounds RPLC is limited. In order to close this analytical gap, two promising advanced separation techniques were investigated for their applicability in water analysis. The serial coupling of RPLC and hydrophilic interaction liquid chromatography (HILIC), and supercritical fluid chromatography (SFC) could both be shown to provide the opportunity to separate a significantly broader polarity range of compounds than RPLC (and can also be combined with mass spectrometric detection). Both techniques allow the parallel and highly reproducible separation as well as the detection of non-polar to very polar compounds. The applicability of the two techniques for TOrCs screening in environmental samples could be verified. The complementarity and orthogonality of both techniques are beneficial for the monitoring of known compounds, but also for the identification of suspects or unknown TOrCs. With the application of these polarity extended separation techniques, it is now possible to monitor water quality more comprehensively and detect more relevant TOrCs, which might pose a risk to environmental and/or human health. This offers the chance of implementing more holistic management strategies, considering a broader spectrum of TOrCs and improving the protection of the environment and drinking water resources.

Zusammenfassung

Das Auftreten von organischen Spurenstoffen in der aquatischen Umwelt stellt eine weltweite Herausforderung dar. Wenngleich die Konzentrationen dieser Substanzen auch vergleichsweise niedrig sind, gibt es große Bedenken über mögliche negative gesundheitliche Auswirkung im Zusammenhang mit Spurenstoffen. Aus diesem Grund haben viele Länder begonnen, Strategien zum Umgang mit Spurenstoffen umzusetzen. Diese Strategien werden häufig auf örtliche Gegebenheiten zugeschnitten und sind an die entsprechen Belastungssituationen, aber auch die Umwelt oder geographische und ökonomische Situationen angepasst. Somit erscheinen verschiedene Strategien sehr unterschiedlich und wenig vergleichbar. Einige Strategien konzentrieren sich auf die Kontrolle von wenigen toxikologisch bewerteten Substanzen, wohingegen andere eine generelle Verminderung des Spurenstoffeintrags in Gewässer verfolgen. Unabhängig von der Ausrichtung der jeweiligen Strategie spielen analytische Techniken eine große Rolle für die Erfassung und Bewertung der Wasserqualität und den Nachweis von Spurenstoffen.

Ziel dieser Arbeit war es, die grundlegenden Prinzipien der vorgeschlagenen oder bereits umgesetzten Managementstrategien zu untersuchen, die jeweiligen analytischen Anforderungen zu identifizieren und zu evaluieren, wie zukünftige analytische Techniken zur Einführung von ganzheitlicheren Managementstrategien und somit zum verbesserten Schutz von Umwelt und Gesundheit beitragen können.

Im ersten Schritt wurden die Managementstrategien aus den Vereinigten Staaten von Amerika (USA), Australien, der Europäischen Union (EU) und der Schweiz untersucht. Die Motivationen der Strategien wurden bewertet und die grundlegenden Paradigmen identifiziert. Es konnte gezeigt werden, dass die Strategien toxizitätsbasierte und/oder emissionsvermeidende Prinzipien nutzen. In den Strategien der USA, Australiens und der EU wurden toxizitätsbasierte Prinzipien in unterschiedlichen Ausprägungen gefunden. Die Strategien der Schweiz und der EU beinhalten (zum Teil) emissionsvermeidende Ansätze. Toxizitätsbasierte Prinzipien sind gut zur Kontrolle einer begrenzten Anzahl risikobewerteter Substanzen geeignet, aber weniger zum Management von unerwarteten Risiken, verbunden mit Substanzgemischen, sowie nicht bewerteten oder unbekannten Substanzen. Emissionsvermeidende Prinzipien können unerwartete Risiken einschränken, jedoch ist eine generelle Eintragsvermeidung von Spurenstoffen in Gewässerkörper, auf Grund der vielfältigen Quellen nur schwer umsetzbar. Eine Kombination von beiden Prinzipien wurde als am besten geeignet eingeschätzt um das Auftreten von Spurenstoffen in Gewässern zu kontrollieren. Toxizitätsbasierte Prinzipien würden zur Kontrolle von bekannten und gefährlichen Substanzen eingesetzt und emissionsvermeidende Prinzipien könnten den Eintrag von Spurenstoffen limitieren und somit unerwartete Risiken verringern.

Zur Überwachung von Spurenstoffen in Gewässern, zum Nachweis der Einhaltung von Qualitätsstandards und zur Identifizierung neuer Spurenstoffe werden in allen Strategien schlagkräftige analytische Methoden eingesetzt. Ein Vergleich der jeweiligen Ansprüche der Strategien an die Analytik zeigte, dass alle Managementstrategien die gleichen analytischen Strategien nutzen (wenn auch in unterschiedlichem Umfang). Dies sind Target-, Suspects und Non-Target-Screening. Die verwendeten analytischen Techniken basieren hauptsächlich auf Umkehrphasen-Flüssigchromatographie (reversed phase liquid chromatography (RPLC)) mit massenspektrometrischer Detektion. Diese Technik ist sehr gut etabliert und eignet sich für die Trennung und den Nachweis von mittel- bis unpolaren Substanzen. Mit zunehmendem Verständnis für die Herkunft und das Umweltverhalten von Spurenstoffen wurde klar, dass das chemische Spektrum von Spurenstoffen größer ist, als der Bereich von Substanzen, die mittels RPLC erfasst werden können. Besondere Limitierungen weist die RPLC für die Trennung von sehr polaren Substanzen auf. Um diese analytische Lücke zu schließen wurden zwei vielversprechende, fortschrittliche Trenntechniken auf ihre Anwendbarkeit für die Gewässeranalytik hin untersucht. Es konnte gezeigt werden, dass die serielle Kopplung von RPLC mit hydrophiler Interaktions-Flüssigchromatographie (HILIC) und die superkritische Fluidchromatographie (SFC), beide mit massenspektrometrischer Detektion, ein signifikant breiteres Spektrum an Substanzen trennen können als RPLC alleine. Die Anwendbarkeit der beiden Techniken für das Screening nach Spurenstoffen in Gewässerproben konnte ebenfalls nachgewiesen werden. Die Komplementarität und Orthogonalität der beiden Techniken bietet große Vorteile für die Überwachung bekannter Substanzen und die Identifizierung unbekannter Substanzen. Die Anwendung beider polaritätserweiterter Trenntechniken erlaubt es die Gewässerqualität umfassender zu bewerten und weitere relevante Spurenstoffe zu identifizieren, die ein Risiko für Umwelt und Gesundheit darstellen können. Dadurch ergibt sich die Chance zur Umsetzung ganzheitlicherer Managementstrategien, die ein breiteres Spektrum an Spurenstoffen betrachten und somit den Schutz der Umwelt und der Trinkwasserressourcen verbessern.

Affidavit

I hereby confirm that this thesis is the result of my own work. I did not receive any help or support from other parties. All sources and / or materials applied are listed and specified in the thesis.

This thesis has not yet been submitted as part of another examination process neither in identical nor in similar form.

Wallerstein, 11th September 2017

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List of Abbreviations

APCI	atmospheric pressure chemical ionization
AWQC	ambient water quality criteria
BPR	back pressure regulator
cAMP	cyclic adenosine monophosphate
CCL	Candidate Contaminant List
CEC	compound of emerging concern
CO_2	carbon dioxide
COMMPS	combined monitoring-based and modelling-based prioritization setting
CWA	Clean Water Act
DBPs	desinfection by-products
DDT	dichloro-diphenyltricholoethane
EDA	effect directed analysis
EDC	endocrine disrupting compounds
EQS	environmental quality standard
ESI	electrospray ionization
EU	European Union
GC	gas chromatography
HRMS	high resolution mass spectrometry
IC	ion chromatography
LC	liquid chromatography
log D	logarithmic octanol-water distribution coefficient
m/z	mass-to-charge
MoA	mode of action
MS/MS	tandem mass spectrometry
NPLC	normal phase liquid chromatography

OECD	Organization for Economic Co-operation and Development
PCBs	polychlorinated biphenyl compounds
ppm	parts per million
QIT	quadrupole ion trap
QqQ	triple-quadrupole
RBD	river basin district
RP	reversed phase
RPLC	reversed phase liquid chromatography
RT	retention time
RTI	retention time index
SDWA	Safe Drinking Water Act
SFC	supercritical fluid chromatography
TMDLs	total maximum daily loads
TOC	total organic carbon
TOF	time-of-flight
TOrCs	trace organic compounds
TP	transformation product
U.S.	United States of America
UCMR	Unregulated Contaminant Monitoring Rule
USEPA	United States Environmental Protection Agency
WFD	Water Framework Directive
WRF	Water Research Foundation
WWTP	wastewater treatment plant

Author Contributions

Chapters of this thesis have been published with slight editorial changes in peer-reviewed journals or books including the following.

Chapter 4:

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Stefan Bieber conducted the literature study and drafted major parts of the manuscript. Shane A. Snyder and Sonia Dagnino drafted the U.S. section of the manuscript. Both and Tanja Rauch-Williams reviewed the manuscript. Jörg E. Drewes reviewed and edited the manuscript and contributed to the structure and discussion.

Chapter 5:

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Stefan Bieber conducted the SFC-MS experiments, evaluated the data and drafted the manuscript. Giorgia Greco established the RPLC-HILIC system and reviewed the manuscript. Sylvia Grosse conducted the RPLC-HILIC experiments and reviewed the manuscript. Thomas Letzel reviewed and edited the manuscript and contributed to the discussion.

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Stefan Bieber performed the SFC-MS experiments, evaluated the data and drafted the manuscript. Steffen Ruppe provided the sample and reviewed the manuscript. Sylvia Große performed the RPLC-HILIC experiments and reviewed the manuscript. Jörg E. Drewes reviewed the manuscript. Thomas Letzel reviewed and edited the manuscript and contributed to the discussion.

Chapter 1 - Introduction

1.1 Trace organic compounds

The presence of organic chemicals in microgram to nanogram per liter concentrations in environmental waterbodies is documented for more than 60 years (Rosen and Middleton, 1955; Middleton and Rosen, 1956; Sallee et al., 1956). In the 1960s, concerns about adverse effects on environmental health posed by trace organic compounds (TOrCs) of anthropogenic origin, namely pesticides emerged, although these occur in only low µg/L to ng/L concentrations (Carson, 1962). About 15 years later, nearly 2,000 compounds had been identified in environmental waterbodies and wastewaters (Keith, 1976a) and prioritization approaches on the basis of occurrence frequency and toxicity were established (Keith and Telliard, 1979). Among frequently detected compounds were halogenated organic compounds and polychlorinated biphenyl compounds (PCBs) (Keith, 1976a, 1981; Jekel and Roberts, 1980; Williams et al., 1984). Continuous advances in analytical instrumentation and techniques, but also an increased knowledge about origin, fate and transformation processes of TOrCs, still allow the identification of new compound classes, such as (residues of) pharmaceutical active compounds or industrial chemicals and to constantly increase the number of detectable compounds (Schlüsener et al., 2015; Richardson and Kimura, 2016; Zahn et al., 2016). Compared to total organic carbon (TOC), which is measured in mg/L, TOrCs constitute only a small portion of the organic chemical composition of water, but represent a broad variety of chemical compounds originating from natural sources as well as human activities. In the European Union (EU), more than 100,000 chemicals are registered and 30,000 to 70,000 of these compounds are in regular use (Schwarzenbach et al., 2006; Loos et al., 2009). A huge proportion of these compounds can potentially enter the aquatic environment (Brack et al., 2017). These compounds reflect the whole spectrum of compounds used in daily life, including biocides/pesticides, industrial chemicals, residues of pharmaceuticals and personal care products, hormones, disinfection by-products (DBPs), other compound classes and an unknown number of metabolites and transformation products (TP) (Schwarzenbach et al., 2006; Richardson and Ternes, 2014). TOrCs can enter the aquatic environment via point and non-point sources. Point sources discharge compounds into the environment at defined locations, like effluents of urban and industrial wastewater treatment plants (WWTPs) (Eggen et al., 2014; Sengupta et al., 2014). Discharges from non-point sources, e.g., combined sewer overflows, run-off from urban and agricultural areas, agricultural drainage pipes, leakages from septic tanks and sewer lines, or aerosol deposition often cannot be located precisely (Neumann et al., 2002; Wittmer et al., 2010; Eggen et al., 2014). The presence of TOrCs in waterbodies impacts environmental and drinking water quality in cases of planned or *de facto* water reuse and pose a potential risk to environmental health and/or human health (Malaj et al., 2014; Rice and Westerhoff, 2014; Drewes and Khan, 2015; Rice et al., 2015).

1.1.1 Effects of TOrCs on environmental and human health

Although TOrCs concentrations in waterbodies are usually very low, there are general concerns about potential adverse effects. Exposure of organisms to chemical compounds can cause the rapid development of severe symptoms (acute effects) or a slow development of symptoms caused by long-term (even life-long) exposure to low concentrations (chronic effects). Acute adverse effects on environmental health are not likely to occur for most compounds, due to sub-therapeutic concentrations present in waterbodies (Enick and Moore, 2007), but long-term exposure of aquatic species might result in chronic adverse effects. Observed effects can be multifold, including e.g. changes in behavior, vitality, reproduction or mortality rate. Some of the compound groups, detectable in waterbodies like biocides, pesticides, hormones and pharmaceuticals are used because of a specific mode of action (MoA), which can target molecular or metabolic pathways of organisms. When emitted into the aquatic environment after intended use or by accident, these MoA may remain active and effect species with identical or similar pathways (Fent et al., 2006; Stehle and Schulz, 2015). An example for such effects has been reported for pharmaceuticals affecting the cyclic adenosine monophosphate (cAMP) dependent signaling pathway (Fabbri and Capuzzo, 2010; Fabbri, 2015). Exposure of marine mussels to propranolol (β-adrenergic antagonist), fluoxetine (selective serotonin-reuptake inhibitor), and carbamazepine (anticonvulsant, mood-stabilizing) affected intracellular cAMP levels, leading to effects similar to those observed in humans altering physical functions (Giltrow et al., 2009; Martin-Diaz et al., 2009; Ericson et al., 2010; Franzellitti et al., 2014). Endocrine disrupting compounds (EDCs) like hormones or other compounds targeting hormone receptors can exhibit serious adverse effects in aquatic species at even very low compound concentrations (Vonier et al., 1996; Kidd et al., 2007). The exposure of fish to ng/L concentrations of 17α-ethinylestradiol can lead to feminization of individuals (Fabbri, 2015), while the exposure of individuals to synthetic progestins can cause masculinization (Runnalls et al., 2013). Species with missing pathways or pathways altered to those normally targeted by a compound, can show unexpected MoA-unrelated effects. Among others, such effects have been reported for fluoxetine in goldfish or diclofenac in vultures. The antidepressant compound fluoxetine causes decreased testosterone levels in goldfish males and impacts reproduction by endocrine disruption, without showing such effects in human (Mennigen et al., 2010). Diclofenac is metabolized in humans through the cytochrome P450 pathway (Bort et al., 1999). In some vulture species, this pathway is altered resulting in the formation of toxic metabolites and sever effects in individuals, when exposed to diclofenac and other non-steroidal anti-inflammatory drugs like ketoprofen (Oaks et al., 2004; Naidoo et al., 2010). Widely described unspecific “side-effects” of exposure to TOrCs are responses to oxidative stress. These result in increased expression and activity of various enzymes, involved in degradation and detoxification pathways like glutathione-S-transferases, glutathione reductase, catalase, superoxide dismutase and others (Brandão et al., 2013;

Carvalho et al., 2014; Fabbri, 2015). Complex mixtures of compounds in waterbodies can cause health effects, although individual compound concentrations are below effect concentrations (Faust et al., 2001; Brian et al., 2005). Such additive or synergistic effects are independent from individual MoA and present a challenge for risk assessment and water quality monitoring (Altenburger et al., 2004; Schwarzenbach et al., 2006). The occurrence of antibiotics in environmental waterbodies at low concentrations is associated with the increasing detection rate of microorganisms carrying antibiotic resistances in various waterbodies (Kümmerer, 2009). As a consequence, antibiotics in waterbodies indirectly pose are risk to environmental and human health through the development of (multi) resistant microorganisms. To detect acquired resistances, (environmental) microorganisms have to be tested for specific antibiotic resistance genes (Rodriguez-Mozaz et al., 2015).

1.1.2 Toxicological assessment of TOrCs

To evaluate adverse effects and/or estimate compound concentrations resulting in effects, studies with suitable organisms (*in vivo*) or cell cultures (*in vitro* approaches) can be conducted. The Organization for Economic Co-Operation and Development (OECD) has published internationally agreed guidelines for the testing of chemicals, which allow to assess potential risks posed by chemicals (OECD, 2016). The guidelines provide about 150 different test methods, including toxicological tests for different endpoints utilizing various species. Individual tests assess acute (e.g. Fish Embryo Acute Toxicity) and chronic effects (e. g. *Daphnia magna* Reproduction Test), but also bioaccumulation, degradability or transformation of chemical compounds for different taxa and trophic levels. The guidelines provide detailed information about required (number of) organisms, testing procedures, data evaluation and statistics for the derivation of no-effect or effect concentrations for specific chemicals on investigated organisms. Further guidance for the assessment of potential risks associated with TOrCs can be provided by national authorities (e.g., USEPA and European Commission). Scientific principles have to be applied with utmost care to achieve reliable and comparable results of high quality (Harris et al., 2014). As an alternative or in addition to toxicity testing, models for the prediction of compound toxicity can be applied. On the basis of a (limited) set of toxicological data, quantitative structure – activity relationship (QSAR) models allow to predict the toxicity of a compound in other species or of related compounds (Zvinavashe et al., 2008; Singh et al., 2014). For the establishment of QSAR models, *in vitro* tests can be used, allowing to reduce the number of tested animals, but the quality of predicted results is strongly depending on the data set. Another alternative is presented in estimation of aquatic toxicity by chromatographic retention in surrogating chromatographic systems (Fernández-Pumarega et al., 2017). All toxicity assessment approaches can result in the determination of no-effect or effect concentrations for a specific endpoint and species. These values can be used to derive more general and protective numerical values.

1.1.3 Protective numerical values

There is a huge variety of numerical values available for TOrCs in many national regulations and guidance documents. Depending on the focus of the individual regulations, these values can either be protective for human health (e.g. drinking water regulations), environmental health (e.g. environmental regulations) or both (e.g., environmental regulations including drinking water source protection). A numerical value does not necessarily have to be executable but can also serve as a health advisory value for orientation. Besides the level of applicability, numerical values can differ in the exposure scenario they cover. For a chronic exposure by a compound, tolerable average concentration values can be applied, while for single-event exposures, acute values could be assigned.

Numerical values can differ strongly regarding protected endpoints, species and exposure durations. In general, there are several levels of numerical values, which can be interrelated (Dieter, 2009). If a certain concentration of a compound is 'naturally' occurring, than this concentration has to be accepted as 'background' concentration. When deriving numerical values for specific compounds, it should be considered that derived quality criteria below the 'background' concentration are hardly applicable. For higher levels of numerical values compound specific information (e.g., data about persistence or toxicity) is required. Since commonly only toxicological data of a limited set of taxa is available, these data have to be extrapolated in order to ensure protection of a broader range of organisms against adverse effects posed by individual compounds. Such predicted no effect concentrations (PNEC) are derived, using the lowest concentration level resulting in observed adverse effects in tested organisms and a safety factor (European Commission, 2003).

According to Dieter (2009) the following levels of values can be distinguished:

- **Precautionary value:** This is the lowest level of values and aiming to prevent exposure from all kinds of adverse effects posed by a compound for current and future events. Ideally, these values represent the 'background' level of contamination.
- **Indicator/orientation value**: For the derivation of these values more compound specific information is required. If measured concentrations in a waterbody exceed a previously determined indicator level than the system enters an 'abnormal' state. As a consequence, measures could be applied, allowing the system to return to a 'normal' state.
- **Value of concern:** In contrast to orientation values, this level of values does not just indicate the possibility of adverse effects, but the probability of adverse effects being induced. Such threshold values can consider the amount of a compound an organism is exposed to and the duration of exposure. A concept proposed by the World Health Organization uses acceptable daily intake values (ADI) (WHO, 2004). These values are based on a life-long average water consumption of 2 liters for a person of 70 kg per day and estimates the tolerable dose of a

compound. If ADI values are not exceeded, the risk of adverse effects from exposure should be minimal. As an additional protective measure, safety factors can be applied on ADI levels (e.g., 10% of the ADI level). An exceedance of values of concern should trigger the implementation of measures to restore acceptable concentrations below the set value.

- **Action value:** An exceedance of these scientifically determined values results in serious concerns for the protected endpoint. Action values are set higher than values of concern, because these relate to an increased risk for adverse effects with increasing time of exposure. Action values can be derived from values of concern and so called ‘interpolation factors’. Action values aim to protect sensitive and/or highly exposed individuals. Measures implemented in case of an exceedance are only meant to limit adverse effects, not to prevent them.

As a response to concerns and potential adverse effects, many countries have started to investigate and implemented strategies for the management of TOrCs, aiming to control potential risks, associated with the occurrence of TOrCs.

1.2 Management strategies for TOrCs

The occurrence of TOrCs in waterbodies and associated (potential) health risks are an international challenge, but drivers and motivations of countries to implement strategies for the management of TOrCs can differ widely. The same applies to the protected endpoints (environmental and/or human health) in environmental waterbodies and/or drinking water (sources) and applied measures, ranging from the control of known, toxicologically assessed TOrCs in waterbodies to a general emission prevention. In addition, there are noticeable differences in the spectrum of compounds, which are addressed by individual programs. Within the universe of TOrCs, there are few well known, toxicologically assessed and regulated compounds, but also emerging contaminants, contaminants of emerging concern (CECs) and completely unknown compounds (Sauvé and Desrosiers, 2014). Emerging contaminants are (new) compounds which have recently been identified. Contaminants of emerging concern are known (or even unknown) compounds (groups), which have not been fully assessed yet, or new information raises concerns about potential health effects. Sauvé and Desrosiers propose to define CECs as unregulated, “*naturally occurring, manufactured or manmade chemicals or materials which have now been discovered or are suspected present in various environmental compartments and whose toxicity or persistence are likely to significantly alter the metabolism of a living being*” (Sauvé and Desrosiers, 2014). Depending on the motivation and paradigms, management strategies can consider all TOrCs, occurring in a waterbody or only some groups, like regulated compounds and/or CECs. As a consequence, individual management strategies can be heterogeneous.

Strategies for the management of TOrCs, which have been proposed and implemented in the United States of America (U.S.), Australia, the EU with emphasis on Germany, and Switzerland have recently been reviewed (Water Research Foundation, 2015). The review was the first part of a project of the Water Research Foundation (WRF) aiming to evaluate current and alternative management strategies for CECs. This phase of the project was carried out by researchers at the University of Arizona (Tucson, Arizona, USA) and the Technical University of Munich under coordination of Carollo Engineers, Inc. (Broomfield, Colorado, USA). The four investigated countries/regions are hardly comparable in size, population (density), climate and economic strength. Thus, management strategies have to address differences in environmental, geographic and economic conditions, but also different occurrence levels of TOrCs.

1.2.1 United States of America

In the U.S., management strategies for TOrCs have been established in two individual regulations for the protection of environmental waterbodies and drinking water. The Safe Drinking Water Act (SDWA), which came into force in 1974, uses enforceable and non-enforceable, nation-wide applicable (quality) standards, based on toxicological assessment to control known hazardous compounds (USEPA, 2014a). New (emerging) contaminants are identified by a rigorous processes called Candidate Contaminant List (CCL) and Unregulated Contaminant Monitoring Rule (UCMR) (USEPA, 2009, 2012a). Drinking water utilities are required to ensure compliance of measured TOrCs concentrations with enforceable standards by implementing adequate mitigation measures. The Clean Water Act (CWA) from 1972, regulates the quality of environmental waterbodies by establishing health based ambient water quality criteria values (AWQC) (USEPA, 2014b). Since these values are intended to protect both environmental and human health, quality criteria can be rather low for e.g. bioaccumulative substances, in order to prevent secondary poisoning. The derivation protocols for quality standards are not harmonized with those used under SDWA and so standards in CWA and SDWA can deviate strongly. If water quality in environmental waterbodies does not reach CWA requirement, these waterbodies are assigned as “impaired”. This results in the establishment of total maximum daily loads (TMDLs). TMDLs are enforceable standards for the maximum amount of a substance that can be loaded to a water body in a single day. All standards under SDWA and CWA have to be implemented by U.S. states, but these may apply more stringent and expansive requirements, than advised by the USEPA.

1.2.2 Australia

Australia suffered from water scarcity and extreme flooding due to extreme weather phenomena in the last two decades. Especially long periods of droughts increased the pressure on drinking water supplies. As a result, alternatives concepts to provide drinking water, including sea water desalination and potable water reuse have been evaluated. To reduce health risks associated with TOrCs in potable reuse, Australia established guidelines for water recycling (NWQMS Phase 1, 2006; NWQMS Phase 2, 2008). The guidelines propose a management concept on the basis of the hazard analysis and critical control point concept (HACCP). This concept evaluates all processes in the water reuse cycle and identifies critical points which could provide potential risks for health or process performance. If critical points are identified, measures are established to minimize risks posed by these points, often utilizing the concept of multiple barriers. Compliance of the process with predefined (performance) goals is monitored thoroughly. In Australian guidelines for water recycling, TOrCs are regarded as potential risk for human health (NWQMS Phase 2, 2008). In order to control these risks, non-enforceable, toxicologically assessed guideline values have been established, providing orientation regarding safe levels of compound concentrations (NWQMS Phase 2, 2008). Enforceable quality standards can be implemented by state governments, but do not necessarily have to be based on these guideline values.

1.2.3 European Union

The EU has established a regulative framework of several directives and regulations, aiming to protect and improve water quality. The Water Framework Directive (WFD) is the core regulation in this framework (European Commission, 2000). It aims to improve the ecological and biological quality of all environmental water bodies and to achieve conditions which show no signs of human influence. Therefore, quality elements contributing to the ecological and chemical status of waterbodies are assessed and, if necessary measures to improve the status have to be implemented. The responsibility to improve water quality in the EU is directed to the member states, which have to assign (trans-national) river basin districts (RBD). Depending on the ecological and chemical status of a RBD, specific measures have to be planned and implemented to reach and maintain a “good” status (program of measures). The definition of good status for different categories of waterbodies and a catalogue of mandatory and supplementary measures are provided in the WFD. TOrCs contribute strongly to the chemical status of waterbodies. With the aim to minimize anthropogenic influence on waterbodies, the WFD intends to avoid the emission of TOrCs in general. To control risks posed by known and toxicologically assessed compounds which can impair waterbodies, acute and chronic environmental quality standards are established (European Commission, 2000, 2008). The emissions of priority hazardous substances, defined under WFD have to be phased out within a given time-frame. In addition, member states are required to identify unregulated environmentally relevant TOrCs, which are discharged in significant amounts. Such river basin specific pollutants, can be identified using different prioritization approaches, including the combined monitoring-based and modelling-based priority setting procedure (COMMPS) (Klein W. et al., 1999; von der Ohe et al., 2011). The WFD, together with other regulations strives to control and reduce the emission of compounds from production over use to disposal (European Commission, 2006a, 2010).

1.2.4 Switzerland

Switzerland currently implements a thoroughly planned and most ambitious strategy for the management of TOrCs (Schweizer Bundesrat, 2016). Starting in 1998, several Swiss research initiatives examined the occurrence and health risk associated with TOrCs and EDCs in particular (Fischnetz, 2004; NRP 50, 2008). Subsequent studies investigated the entrance pathways for TOrCs into the aquatic environment (Gälli et al., 2009). Different strategies for the control of TOrCs, including source control and end-of-pipe approaches were evaluated (Abegglen et al., 2009; Gälli et al., 2009). WWTP effluents were identified as a major emission source for TOrCs. Following the maxim, all TOrCs occurring in waterbodies present a potential risk for environmental health, the emission of TOrCs into the aquatic environment should be minimized. As a consequence, the Swiss parliament decided to upgrade wastewater treatment in 100 of the 700 existing WWTPs (Eggen et al., 2014). This measure is expected to reduce the discharge of TOrCs into waterbodies by 50% and increase wastewater treatment costs by approximately 6% (Eggen et al., 2014). The costs for implementation and operation will be refinanced by an additional fee for wastewater producers, following the polluter-pays principle (BAFU; Abteilung Wasser, 2012).

To monitor the compliance with the requirements of management strategies, robust and reliable analytical techniques are required.

1.3 Analytical techniques for the identification and monitoring of TOrCs

The identification and monitoring of TOrCs in waterbodies is mainly conducted using gas chromatography (GC) and (high performance) liquid chromatography (LC), usually coupled to mass spectrometry (MS) for a mass selective detection (Koutsouba et al., 2003; Vanderford and Pearson, 2003; Vieno et al., 2005; Westerhoff et al., 2005; Schymanski and Singer, 2014). From the beginning, knowledge about the occurrence of TOrCs was strongly related to advancements in analytical techniques. GC which is older than LC was initially used to detect TOrCs in waterbodies (James and Martin, 1952). The development of the electron capture detector allowed the detection of chlorinated compounds like dichlorodiphenyltrichloroethane (DDT) and PCBs (Lovelock, 1958, 1974). The detection of DDT in the environment, which was related to a decrease in bird populations in the 1950s and 60s led to the publication of Rachel Carson's *Silent Spring* (Carson, 1962). This is considered to be a main driver for the development of the environmentalist movement in the following years. In the late 1970s, already approximately 2,000 compounds had been identified in waterbodies using GC-MS (Keith, 1976a, 1976b). Although GC is well suitable for the separation of volatile and non-polar compounds, it soon became obvious that the technique is only applicable for the separation and detection of a minority of the organic compounds with hydrophobic properties (5-20%), present in the aquatic environment (Keith, 1976a). For the detection and identification of less volatile and more polar TOrCs, LC-MS techniques were established. The use of reversed phase (RP) stationary phases with electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) allowed to significantly extend the range of detectable TOrCs in waterbodies. Still major efforts are taken to optimize analytical instrumentation and techniques and to establish analytical strategies for the quantification of known compounds and the identification of unknown compounds, aiming to receive a more comprehensive view on relevant TOrCs in the aquatic environment.

1.3.1 Advances in instrumental analysis

1.3.1.1 Liquid chromatography

RPLC with MS detection, relying on C18 stationary phases is currently the most used separation technique for the identification and monitoring of TOrCs (Vanderford and Pearson, 2003; Vieno et al., 2005; Deeb et al., 2017). This technique is robust, well established and ideal for separation and detection of non-polar compounds (logarithmic octanol-water distribution coefficient at a pH of 7 (log D (pH 7)) > 0). Increasing knowledge about the chemical nature of trace organic compounds and transformation processes in water samples led to growing awareness about the presence of more polar compounds in environmental samples. These compounds present a major challenge in water analysis, due to insufficient retention in RPLC resulting in ineffective ionization (Reemtsma et al., 2016). The introduction of polar modified C18 stationary phases, allowed to shift the accessible range of compounds towards medium polar compounds (log D (pH 7) > -2.5) (Periat et al., 2013; Reemtsma et al., 2016). Such polar modification can be achieved by polar end-capping, the addition of polar groups in the non-polar alkyl chains of the stationary phase, or the combination of different retentive materials in a stationary phase. Examples for such mixed mode LC phases are the combination of RP and weak anion-exchanging material (Balkatzopoulou et al., 2015) or RP with anion and cation-exchangers (Montes et al., 2017). Separation techniques like ion-pair LC, ion chromatography (IC), normal phase liquid chromatography (NPLC) or hydrophilic interaction liquid chromatography (HILIC) are more suitable for the separation of very polar compounds (Zhu et al., 1999; Loos and Barceló, 2001; Shao et al., 2002; Kahsay et al., 2014). But in contrast to RPLC these techniques lack the capability of separating non-polar compounds and/or are not suitable for the analysis of aqueous samples. Ideally, chromatographic techniques should provide the separation of non-polar to very polar compounds in a single run. Such systems can be realized by the combination of stationary phases in two-dimensional chromatography. Some combinations, which might provide benefits in the analysis of TOrCs are summarized in the following:

RPLC/RPLC: The use of a RP stationary phases with different mobile phase compositions in both dimension or different stationary phases with the same mobile phase composition can provide a certain degree of orthogonality (Dugo et al., 2008). This can be useful to resolve a limited number compounds with comparable polarity, which could not be achieved in one dimensional separations.

IC/RPLC: The combination of ion chromatography is well suitable for the separation of charged (hydrophobic) molecules, like organic acids, fatty acids and proteins from complex samples (Brudin et al., 2010; Zhu et al., 2012; Bang and Moon, 2013).

NPLC/RPLC: The combination of NPLC and RPLC offers a high degree of orthogonality and is suitable for the separation of uncharged molecules in a broad hydrophobicity range (Dugo et al., 2004). Due to the general immiscibility of mobile phases in NPLC and RPLC, the transfer of analytes from the first to the second dimension requires special considerations. Couplings of NPLC and RPLC have been realized using miscible mobile phases in NPLC and RPLC, a strong dilution of NPLC solvents when entering the RPLC columns, or a solvent evaporation device on the transition from NPLC to RPLC (Dugo et al., 2008; Tian et al., 2008).

HILIC/RPLC: The solvents used in HILIC and RPLC are miscible and so the combination of RPLC and HILIC or vice versa is an alternative to NPLC/RPLC with a high degree of orthogonality. HILIC/RPLC is suitable for the separation of hydrophilic to hydrophobic compounds and depending on the utilized HILIC phases also for charged molecules (Xing et al., 2012; Greco and Letzel, 2013).

1.3.1.2 Supercritical fluid chromatography

Another promising separation technique is presented by supercritical fluid chromatography (SFC). SFC was introduced in 1962 as alternative technique to GC for the separation of thermo-labile analytes (Klesper et al., 1962). Due to some misunderstandings and unfulfillable expectations, it took more than 20 years for SFC to gain acceptance as a serious separation technique (Saito, 2013; Berger, 2014; Bieber and Letzel, 2015a). Nowadays, it is a well-established technique for the (preparative scale) separation of chiral molecules. With the introduction of a new generation of analytical scale instruments in 2010 and 2012 with improved robustness and reliability, the interest in SFC increased. Instruments in analytical and preparative scale are now commercially available from several vendors (Bieber and Letzel, 2015b). The instrumentation of SFC is comparable to that known from LC, but the technique combines benefits of both, GC and LC. The mobile phase in SFC is mainly comprised of pressurized carbon dioxide (CO_2) and a small percentage of organic solvents which are used to modify the elution strength of the mobile phase. The pressure of the mobile phase is usually set above the critical pressure (73.8 bar) and temperatures between 20°C and 50°C. The critical temperature of CO_2 is at 31°C, but a temperature above the critical value is not necessary for successful separations (Tarafder et al., 2014; Lesellier and West, 2015). Depending on the set pressure and temperature values, the mobile phase will reach a liquid or supercritical state. In separations, it is important to maintain a preset thermodynamic state of the mobile phase. Therefore, a back pressure regulator (BPR) is located at the end of the chromatographic system. The BPR allows to control the overall pressure in the system and provides a certain pressure at the end of the chromatographic column. This prevents the mobile phase from changing its thermodynamic state within the chromatographic column (Bieber and Letzel, 2015c; Lesellier and West, 2015). The density of the mobile phase is comparable to a liquid, but the viscosity is on the level of gaseous media. This allows very efficient and fast separations and the use of small particle diameters in packed columns. These unique characteristics of the mobile phase make SFC an interesting alternative separation technique (Bieber and Letzel, 2015c; Lesellier and West, 2015). The density of the mobile phase in SFC separations is dependent from the applied pressure. This compressibility is usually not observed in LC separations and requires special attention in SFC. The density of the mobile phase can be influenced by several (adjustable) factors and affect selectivity and retention (Lesellier and West, 2015; Bieber and Letzel, 2017). All stationary phases, known from LC can be used for SFC separations, but basic retention mechanisms in SFC are not completely understood, yet (Lesellier, 2009). As a consequence, method development in SFC is commonly started with the screening of different stationary phases. Classification approaches on the basis of physico-chemical properties of stationary phases allow to select a set of phases for screening and to speed up method development (Bieber and Letzel, 2015d; Lesellier and West, 2015). SFC can easily be coupled to mass spectrometers, providing the chance to apply the separation technique in many analytical fields, including the screening of TOrCs in aqueous samples.

1.3.1.3 Mass spectrometry

MS is the most commonly used detector for the analysis of TOrCs (Richardson, 2012; Richardson and Kimura, 2016). Depending on the chosen strategies (quantitative or qualitative analytics), different requirements concerning the quality of MS data and subsequent data evaluation have to be considered. Important parameters are the sensitivity, the mass accuracy and the resolution of MS instruments, which are dependent of the type of instrument. Highest sensitivity can be achieved with triple-quadrupole instruments (QqQ) and quadrupole ion traps (QIT) (Krauss et al., 2010). Orbitrap and time-of-flight instruments (TOF) provide a lower (constantly improved) sensitivity, but a significantly better mass accuracy (approximately 2-3 ppm accuracy) and higher resolution, than QqQ or QIT instruments (approx. 50 ppm). Typical resolutions of Orbitrap instruments are in the range of 100,000 at 300 to 400 mass-to-charge (m/z) and around 10,000 to 20,000 for TOF-MS. The resolution of QqQ and QIT is commonly lower than those obtained with other instruments, but usually depending on the mass range and the scan speed (Krauss et al., 2010). Tandem mass spectrometry (MS/MS) can provide structural information of compounds, in addition to mass information. Besides QqQ and QIT instruments, hybrid instruments, such as quadrupole/TOF (Q-TOF) and linear ion trap/orbitrap (LTQ

Orbitrap) can be used for MS/MS experiments. All above mentioned instrument types can be used for target screening which is conducted for quantitative monitoring of known compounds. Accurate mass and high resolution MS (HRMS) allow to determine the sum formula of a detected compound. As a consequence, HRMS are more suitable for suspects and non-target screening than QqQ and QIT, but yet do mostly not provide the sensitivity and linearity as provided by QqQ instruments.

1.3.2 Analytical strategies

Analytical strategies can be used for the monitoring of environmental concentrations of known compounds or the identification of expected or unknown compounds (Aceña et al., 2015; Letzel et al., 2015). For the quantitative analysis of known compounds, target screening is applied. For this strategy reference standards (stable isotope-labelled) of target compounds are required. These standards are added to the sample prior to sample pretreatment procedures and used to validate and quantify unlabeled compounds, occurring in analyzed samples. Suspects and non-target screening are applied to identify so far only expected or unknown compounds in samples. Suspects screening uses information about e.g. the origin of a sample to create a list of expected compounds. Therefore, masses of expected compounds are used for multiple reaction monitoring (MRM) with tandem-MS (Letzel et al., 2015). Data can be used for the search for matching entries in various sources as literature and analytical or MS databases. For the verification of the presence of a compound in a sample, reference material is required, resulting in target-screening. Non-target screening is applied for the identification of unknown compounds in samples. Such compounds are not contained in any database. As a consequence, analytical data, like monoisotopic mass, fragmentation behavior in MS/MS experiments or hydrophobicity can be used, to gain further information about compound characteristics (Letzel et al., 2015). This information can be condensed to hypothetical chemical structures, which have to be validated using reference material.

Chapter 2 - Research significance and hypotheses

TOrCs are an international challenge of the water sector and their impact on the environment cannot be foreseen so far. Many countries have started to implement strategies to manage TOrCs in the man-made water cycle, which are adjusted in order to consider local conditions, TOrCs occurrence, water quality, and drinking water supply conditions. As a consequence, strategies are heterogeneous, although countries share comparable concerns. The protected endpoints and the implemented measures differ strongly. Thus, transferring a national management strategy into another watershed or country might not be reasonable or possible. In order to identify more widely applicable concepts, the underlying basic principles of these strategies have to be revealed. Management strategies are implemented to respond to the presence of TOrCs, so analytical techniques play an important role, providing information about TOrCs occurring in waterbodies. Since management strategies can only address compounds, which are detectable by analytical techniques, potential limitations in instrumental analytics would prevent a more holistic management of TOrCs. Reemtsma et al. (2016) recently highlighted that currently used analytical techniques are not capable of separating and detecting polar and mobile TOrCs (Reemtsma et al., 2016). Due to the good solubility of polar and very polar compounds in water, it is very likely that a significant number of such compounds is present in the aquatic environment. Without suitable analytical techniques, an assessment of these compounds and an adjustment of management strategies is not possible. Thus, alternative separation techniques, allowing to extend the spectrum of separable and detectable compounds towards more polar compounds are required. Such techniques would offer a broader view on the spectrum of chemical compounds present in the aquatic environment and allow to establish more holistic management strategies for a comprehensive protection of environmental and/or human health.

The objectives of this dissertation are fourfold:

- To identify the underlying principles of currently proposed or implemented management strategies for TOrCs by conducting a review on approaches in different countries,
- To categorize analytical tools, used by these principles in order to assess the potential impact of analytical advances on management strategies,
- To evaluate the potential of two advanced separation techniques, namely RPLC-HILIC/TOF-MS and SFC/TOF-MS for the analysis of TOrCs compared to commonly used techniques, and
- To investigate the benefits of using the two complementary separation techniques for the identification of TOrCs in parallel.

The following research hypotheses will be tested in this thesis in order to complete the objectives:

Hypothesis #1: Management strategies for TOrCs consist of toxicity- and/or emission avoidance-based principles.

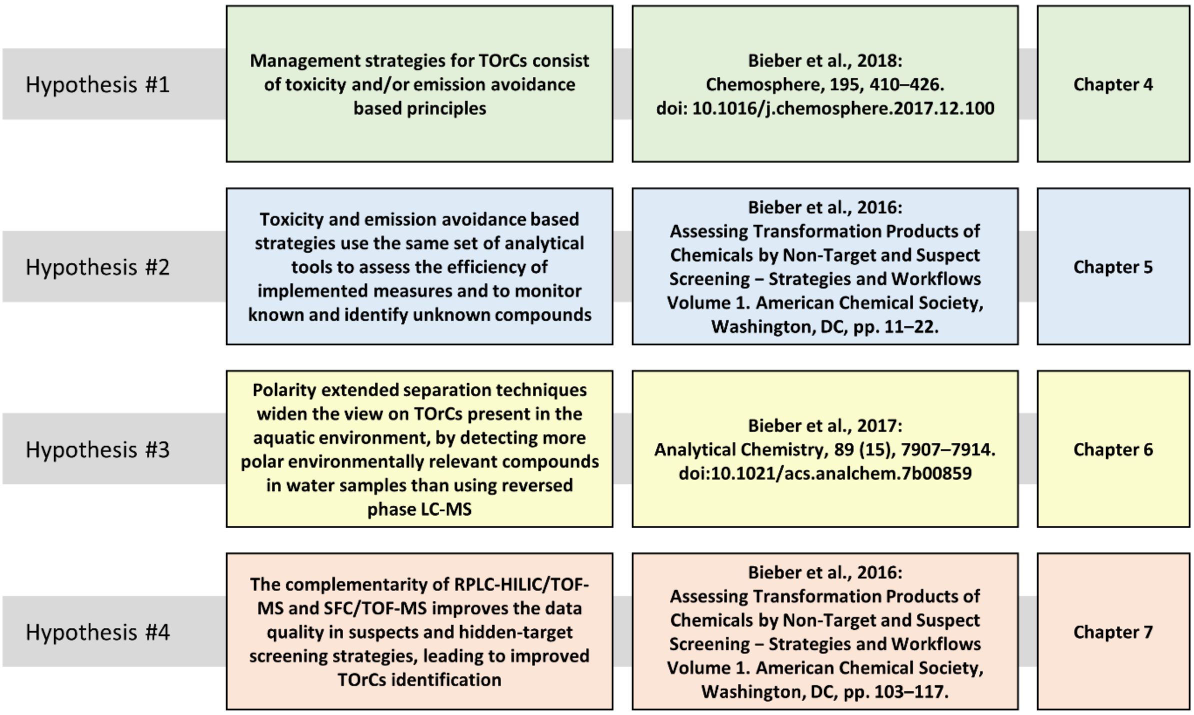
Hypothesis #2: Toxicity- and emission avoidance-based strategies use the same set of analytical tools to assess the efficiency of implemented measures and to monitor known and identify unknown compounds.

Hypothesis #3: Polarity extended separation techniques widen the view on TOrCs present in the aquatic environment, by detecting more polar environmentally relevant compounds in water samples than reversed phase LC-MS.

Hypothesis #4: The complementarity of RPLC-HILIC/TOF-MS and SFC/TOF-MS improves the data quality in suspects and hidden-target screening strategies, leading to improved TOrCs identification

2.1 Structure of the dissertation

This dissertation is structured on the basis of the four research hypotheses (Figure 2-1). The following chapter gives a short summary of the methodological approaches. In Chapter 4 to 7 the research hypotheses are tested. The chapters contain a summary of the scientific background, materials and methods (only in Chapter 6 and 7), the results of the conducted experiments and a discussion.



Hypothesis #1	**Management strategies for TOrCs consist of toxicity and/or emission avoidance based principles**	**Bieber et al., 2018: Chemosphere, 195, 410–426. doi: 10.1016/j.chemosphere.2017.12.100**	**Chapter 4**
Hypothesis #2	**Toxicity and emission avoidance based strategies use the same set of analytical tools to assess the efficiency of implemented measures and to monitor known and identify unknown compounds**	**Bieber et al., 2016: Assessing Transformation Products of Chemicals by Non-Target and Suspect Screening – Strategies and Workflows Volume 1. American Chemical Society, Washington, DC, pp. 11–22.**	**Chapter 5**
Hypothesis #3	**Polarity extended separation techniques widen the view on TOrCs present in the aquatic environment, by detecting more polar environmentally relevant compounds in water samples than using reversed phase LC-MS**	**Bieber et al., 2017: Analytical Chemistry, 89 (15), 7907–7914. doi:10.1021/acs.analchem.7b00859**	**Chapter 6**
Hypothesis #4	**The complementarity of RPLC-HILIC/TOF-MS and SFC/TOF-MS improves the data quality in suspects and hidden-target screening strategies, leading to improved TOrCs identification**	**Bieber et al., 2016: Assessing Transformation Products of Chemicals by Non-Target and Suspect Screening – Strategies and Workflows Volume 1. American Chemical Society, Washington, DC, pp. 103–117.**	**Chapter 7**

Figure 2-1: Relation between tested hypotheses, publications and chapters of this thesis.

Chapter 4 focuses on the management strategies, proposed and implemented in various countries to control TOrCs in waterbodies. Examples from the U.S., Australia, the EU (with special focus on Germany) and Switzerland are investigated and underlying principles are compared. In Chapter 5, mitigation measures for TOrCs are summarized and the analytical tools, which are required for individual management strategies are classified. The suitability and potential of RPLC-HILIC and SFC coupled to TOF-MS for the separation and detection of non-polar to very polar TOrCs in water samples is evaluated in Chapter 6. Benefits for the identification of TOrCs by using both complementary separation techniques in parallel are investigated in Chapter 7. In Chapter 8, the results of the conducted studies and the hypothesis tests are summarized. Chapter 9 provides conclusions and gives an outlook on future developments, challenges and research needs.

Chapter 3 - Materials and methods

Literature studies were conducted to collect information about management strategies in the U.S., Australia, the EU with focus on Germany, and Switzerland. Peer-reviewed articles and book chapters, official regulations and guideline documents from targeted countries and scientific reports of national agencies served as sources for the literature studies. The motivations and drivers for the implementation of management strategies were investigated for each country/region and core principles were extracted. Individual strategies were compared and assessed on a common basis. Analytical strategies and workflows, which are required for individual management strategies were evaluated and categorized into target, suspects and non-target approaches, a classification scheme which is widely used in (environmental) analytics (Letzel et al., 2015; Schymanski et al., 2015).

For the evaluation of polarity extended separation techniques, a serial coupling of RPLC and HILIC and a SFC system was used. The RPLC-HILIC coupling was set up as described by Greco et al. (2013). Both systems were coupled to an ESI-TOF-MS. The accessible polarity range of both systems was determined by analyzing 274 chemical compounds in a polarity range from log D (pH 7) = -7.71 to +7.67. The same set of compounds was used to study the inter- and intraday reproducibility of separations. Retention times (RT) and detected mass were collected in an in-house database which was the basis for a suspects/hidden-target screening in a pre-concentrated WWTP effluent sample. The influence of the matrix on the ionization in both techniques was investigated by the comparison of MS signal heights of equal amounts of compounds analyzed in low and high matrix containing samples. The applicability of the two techniques for target- and suspects screening was investigated, using a pre-concentrated water sample of the Rhine River. For the target screening, stable-isotope labelled internal standard compounds were added to the sample. Further details about experimental details are provided in the experimental and material and methods sections in Chapters 6 and 7.

Chapter 4 - Management strategies for trace organic chemicals in water – a review of international approaches[1]

To ensure an appropriate management of potential health risks and uncertainties from the release of trace organic chemicals (TOrCs) into the aqueous environment, many countries have evaluated and implemented strategies to manage TOrCs. The aim of this study was to evaluate existing management strategies for TOrCs in different countries to derive and compare underlying core principles and paradigms and to develop suggestions for more holistic management strategies to protect the environment and drinking water supplies from the discharge of undesired TOrCs. The strategies in different industrial countries were summarized and subsequently compared with regards to three particular questions: 1) Do the approaches different countries have implemented manage all or only specific portions of the universe of chemicals; 2) What implementation and compliance strategies are used to manage aquatic and human health risk and what are their pros and cons; and 3) How are site-specific watershed differences being addressed? While management strategies of the different countries target similar TOrCs, the programs differ in several important aspects, including underlying principles, the balance between aquatic or human health protection, implementation methods, and financing mechanisms used to fund regulatory programs.

This publication tested the hypothesis that all management strategies for TOrCs consist of toxicity- and/or emission avoidance-based principles.

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[1] Bieber, S.; Snyder, S. A.; Dagnino, S.; Rauch-Williams, T.; Drewes, J. E., 2018. Management strategies for trace organic chemicals in water – A review of international approaches. Chemosphere 195, 410–426. doi: 10.1016/j.chemosphere.2017.12.100

Chapter 4 - Management strategies for trace organic chemicals in water – a review of international approaches

4.1 Introduction

The presence of natural and synthetic trace organic compounds (TOrCs) in the aqueous environment is well documented (Luo et al., 2014; Postigo and Barceló, 2015) and commonly occur at the microgram per liter (µg/L) to sub-nanogram per liter (ng/L) or picogram per liter (pg/L) concentration range (Schwarzenbach et al., 2006). Detectable anthropogenic TOrCs in aqueous samples reflect the whole universe of chemicals used in daily life, including pesticides, herbicides, industrial chemicals, pharmaceuticals, personal care products, and their transformation products. TOrCs can be further classified into regulated compounds, which represent only a small portion of TOrCs and unregulated compounds. Among unregulated TOrCs, there are recently detected and yet not fully assessed compounds (emerging contaminants) which may pose a risk to environmental and/or human health (contaminants of emerging concern (CEC)) as well as undetected and unknown compounds (Sauvé and Desrosiers, 2014). Another important group are transformation products (TP), which originate from regulated and unregulated TOrCs (Cwiertny et al., 2014). Most chemicals used in daily life are rather hydrophilic and enter the aquatic environment through various pathways. TOrCs enter the aquatic environment via point sources like wastewater effluent discharges from municipal and industrial wastewater treatment plants or combined sewer overflows (CSOs) and via non-point sources like urban or agricultural run-off, agricultural drainage pipes, leakages from septic tanks and sewer lines, or aerosol deposition (Neumann et al., 2002; Wittmer et al., 2010; Eggen et al., 2014).

Adverse effects on environmental health have been documented for several TOrCs (de Zwart et al., 2006; Schäfer et al., 2011), however, the overall health impact of anthropogenic TOrCs is largely unknown. Besides documented adverse effects from individual chemicals, there is concern about unknown or not toxicologically assessed compounds (Schwarzenbach et al., 2006). Several studies on chronic exposure to single TOrC reported adverse effects on aquatic organisms (Fent et al., 2006; Malaj et al., 2014). Many chemicals in daily use possess specific modes of action, which remain active when these chemicals enter the aquatic environment. This can cause health effects comparable to those originally intended or unexpected effects in aquatic organisms for instance effects related to oxidative stress (Fabbri, 2015). Assessing potential risks from all known TOrC is hardly possible. A large percentage of chemicals in water bodies will remain unknown and moreover, mixtures of chemicals might result in effects that are not triggered by individual compounds (Faust et al., 2001; Fent et al., 2006). Since surface or groundwater supplies also serve as source water for drinking water production, some TOrCs have the potential to adversely affect drinking water quality (Stackelberg et al., 2004). This is of particular concern, where *de facto* (unplanned) reuse of water through discharge of treated wastewater effluents upstream of drinking water intakes or planned potable water reuse is practiced (Rice and Westerhoff, 2014; Drewes and Khan, 2015; Rice et al., 2015).

Depending on the usage of individual chemicals, specific chemical classes exhibit seasonal variability at elevated concentrations (e.g., insecticides, herbicides) (Konstantinou et al., 2006; Schäfer et al., 2011), while other classes are used and detected with lower concentration variations (e.g., personal care products, pharmaceuticals) (Schäfer et al., 2011). The preference for certain compounds can also differ by geographic region, per-capita water consumption, or prescription practices. A prominent example is the analgesic drug diclofenac, which is a widely prescribed and over-the-counter drug in European countries and as a result frequently detected in wastewater treatment plant effluents and surface waters at concentrations in the low microgram per liter range (Zhang et al., 2008). Observed concentrations in the United States are generally lower, which is likely an effect of different prescription practices or drug application. In addition, different per-capita drinking water consumptions can result in different dilution ratios in the generated municipal wastewater. These differences and fluctuations in compound usage result in significant variability in occurrence of TOrC in water bodies across the globe. Based on known chemical usage data, combined with data describing discharge and flow of receiving streams, several studies have proposed to model and predict environmental concentrations of selected TOrC (Schulze and Matthies, 2001; Schowanek and Webb, 2002; Anderson et al., 2004). These tools are well-established for known TOrC with sufficient fate and transport data and are being used to assess their site-specific toxicological risk (Lienert et al., 2007; Schüürmann et al., 2011).

To ensure an appropriate management of potential health risks and uncertainties, many countries have evaluated and implemented strategies to manage TOrC in water bodies. Among implemented strategies, comparable motivations and paradigms can be identified (Bieber et al., 2016a). However, these national strategies have been adjusted to consider specific local conditions, including prevalence in occurrence, individual degrees of water quality impairment, and site-specific drinking water supply conditions. The focus of this study was to evaluate existing management strategies for TOrC in selected countries, who had established noticeable management programs or program elements. The aim of this investigation was to derive and compare underlying core principles and paradigms, to assess their effectiveness, and to develop suggestions for more holistic management strategies to protect the environment and drinking water supplies from discharge of TOrC.

4.2 Methods: Case studies and evaluation criteria

The management of TOrC for environmental and human health protection poses specific challenges to law makers, regulators, watershed managers, and water treatment providers around the globe. This study investigated how different industrialized countries address the following challenges:

1. The extent to which strategies consider different groups of TOrC: Considering the current state of knowledge, adverse effects, posed by TOrCs are only partially known and quantifiable for a rather short list of known chemicals. The vast number of TOrC is too large to conduct traditional compound-by-compound regulatory risk assessments for each chemical and their transformation products. Beside known and detectable TOrC, the universe of TOrC also includes chemicals which are known to exist but are not yet characterized (so-called "known unknowns") or represents chemicals that might be present but have not yet been identified (so-called "unknown unknowns").

2. Implementation and compliance strategies specific to the management of human and aquatic health risk: Finding the proper balance between science-based water quality goals, resources available, and implementable policy requirements can be approached differently.

3. The ability to adapt the implementation of management strategies to different site-specific watershed conditions. The fact that chemical occurrence is watershed- and site-specific requires regional-specific management approaches giving proper recognition to local conditions.

Four countries and the European Union (EU), all with noticeable ongoing initiatives regarding management strategies for TOrC in the aquatic environment, were selected for this study: The United States of America (USA), Australia, Germany, and Switzerland. These countries were selected for this study because they differ not only in their philosophy in managing chemicals in the aquatic environment and their underlying regulatory frameworks, but also in their demographic, geographic and economic boundary conditions (Table 4-1). The average population density of investigated countries ranged from 3/km^2 (Australia) to 232/km^2 (Germany). The two largest countries of the study (by land area), Australia and the USA, were also those exhibiting the lowest average population density. Regarding the distribution of urban settlements in Australia and the USA, both countries are heterogeneously populated including densely populated urban areas along the coastal regions, while inland areas for instance in Australia are sparsely populated. Switzerland, with only a fraction of the size of Australia or the U.S., exhibits similar regional differences in population density between alpine and lowland areas. The population density in the heavily populated areas of northern Switzerland is 558 people/km^2 compared to the average of 207 people/km^2 for the entire country (Table 4-1).

Among all countries, surface water is the major source of drinking water, followed by groundwater. Water desalination of brackish water and seawater, planned potable reuse and rainwater collection currently are only of minor importance as a source for drinking water. The gross domestic products (GDP) of Switzerland and the U.S. are at a comparable level, as well as the GDPs of Europe and Australia. Economic strength can be an important factor for the implementation of measures to control TOrC, because monitoring programs, administrative oversight, and technical management approaches for the reduction of CECs require financial resources.

Table 4-1: Specifics of investigated countries and political union

Country	Population (2014) in millions	Area [km^2] [e]	Population density [People/km^2] [e]	Gross Domestic Product per Capita in 2014 [US-$] [f]	Annual freshwater withdrawal in 2008-2012 (billion m^3)[g]	Total water withdrawal per capita [m^3/inhabitant/year][g]
EU	506.9[b]	4,324,782[d]	117	35,849	250[h]	-
USA	318.9[a]	9,147,420[c]	35	54,630	419	1,543
Germany	80.9[a]	348,540[c]	232	45,802	33	410
Australia	23.5[a]	7,682,300[c]	3	43,930	16	824
Switzerland	8.2[a]	39,516[c]	207	57,235	2	250

[a] data.worldbank.org/indicator/SP.POP.TOTL

[b] ec.europa.eu/eurostat/web/population-demography-migration-projections/population-data/database

[c] data.worldbank.org/indicator/AG.LND.TOTL.K2

[d] https://www.cia.gov/library/publications/the-world-factbook/fields/2147.html

[e] data.worldbank.org/indicator/EN.POP.DNST

[f] data.worldbank.org/indicator/NY.GDP.PCAP.PP.CD http://www.imf.org/

[g] Source: Surface water/groundwater/desalination - www.fao.org/nr/water/aquastat/data/query/index.html?lang=en

[h] estimated EU wide withdrawal on withdrawal data of 24 of 28 EU member states, obtained from [g]

4.3 National strategies to manage TOrCs in the aquatic environment

Since national management strategies for TOrCs include different chemicals and chemical groups, different views exist regarding what is and what is not considered a CEC and whether to include or exclude already regulated chemicals in this definition among the targeted countries. Since this study is intended to investigate management strategies for all anthropogenic chemicals, which can be detected in the aquatic environment and drinking water at µg/L to ng/L concentration levels, the term 'TOrC' will subsequently be used.

4.3.1 United States of America

4.3.1.1 Socio-political background

The United States of America is a federal republic that includes 50 states, one federal district, and six territories which includes highly variable geographies from temperate rain forest to parched arid deserts. This large geography also experiences tremendous variation in natural water deposition, resulting in torrential flooding in some regions to prolonged droughts in others. About 64% of the drinking water used in the U.S. comes from surface water, with groundwater accounting for the remaining 36% (USGS, 2017). Nearly 153,000 drinking water systems (all categories included) are located across the U.S., with the majority of community water systems supplied by groundwater (91%), however, the majority of the U.S. population (71%) is supplied by community water systems that use surface water (USEPA, 2013). Wastewater is treated in 16,000 publicly-owned wastewater treatment plants in the United States and its territories (USEPA, 2013). Because of the large geography and diversity of land usage, the USA has established federal laws for the protection of water resources and drinking water that must be met by all U.S. states. However, individual states may elect to apply additional regulations and/or more stringent regulations than those mandated by the federal government.

4.3.1.2 Development of environmental regulations for water

As early as 1914, the USA established regulations that began to link pollution in rivers to adverse health impacts to people, particularly for bacteria. Therefore, national standards were developed for the disinfection of drinking water using chlorine. The Water Pollution Control Act was passed in 1948 and was driven by local concerns as at the time regulatory actions were still made by individual states. The drivers for this act were principally related to human health and created a comprehensive set of water quality programs that also provided some financing for state and local governments. In 1965, the Water Quality Act was designated to adopt water quality standards only for receiving waters and required states to issue water quality standards for interstate waters, and authorized the newly created Federal Water Pollution Control Administration to set standards where states failed to do so.

On the basis of the Water Pollution Control Act, the Clean Water Act was introduced in 1972, regulating effluent discharges and water quality of the receiving aquatic environment. In 1974, the Safe Drinking Water Act came into force, which is the primary mechanism for ensuring drinking water quality is compliant with the treatment standards required. These regulations are administered by the U.S. Environmental Protection Agency (USEPA). The USEPA is responsible for setting national criteria, standards, and regulations that then will be enforced by the agency and states (USEPA, 2016a).

4.3.1.3 Existing approaches for regulated TOrCs

The U.S. federal system uses a combination of enforceable water quality standards, non-enforceable secondary standards, water quality criteria, water quality goals, guidelines, and health advisories to protect both human and environmental health. Enforceable water quality standards are generally derived following toxicity-based approaches, in contrast to non-health based secondary standards addressing aesthetic (taste and odor), cosmetic (color) and technical effects (e.g. corrosion potential) (USEPA, 2017a). Some programs go beyond the immediate protection of water quality and require toxicological evaluations of commercial products before and after production. This is especially true for pharmaceuticals, pesticides, and high-production volume chemicals (Snyder, 2014). The U.S. Food & Drug Administration (FDA) requires that all pharmaceuticals in the USA undergo extensive animal testing followed by clinical human trials before approval can be considered for marketing for human health treatment. In addition, the FDA requires eco-toxicological testing when a drug is reasonably expected to occur in water and/or soil at 1 µg/L or 100 µg/kg, respectively (FDA, 1998; Snyder et al., 2003). Pesticides are regulated (in part) under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), which is intended to "*provide federal control of pesticide distribution, sale, and use*" (USEPA, 2016b) to protect human health and the environment from unreasonable adverse effects of pesticides. All pesticides used in the USA must be registered by the USEPA, their registration is specific to a certain use and/or crop (USEPA, 2017b). The manufacturer is required to submit an application that includes health, environmental and safety data. In addition, the USEPA can regulate the amount of pesticides allowable in receiving waters and drinking waters through the CWA and SDWA, respectively.

One of the most comprehensive chemical management programs in the USA by regulating the production, use, and disposal (release) of new and existing commercial chemicals to pose unreasonable risk to human and environmental health, is the Toxic Substances Control Act (TSCA). Thus, manufacturers must submit pre-manufacturing notification to the USEPA for chemicals to be produced and/or imported. Some chemicals are excluded from TSCA, such as pesticides and pharmaceuticals, which are covered under previously mentioned legislature and chemicals to be used solely for R&D purposes. Under TSCA authority, the USEPA also administers the Pollution Prevention Act, which is a precautionary approach that seeks to reduce pollution by focusing on source reduction processes through cost-effective changes in production, operation, and raw materials (such as green chemistry substitutes and practices).

4.3.1.4 Water quality protection from TOrCs and consideration of new candidates for regulations

Despite the wide array of various programs, the most critical regulations for maintaining water quality in the USA are the SDWA and CWA. The SDWA establishes primary and secondary standards for potable water that are part of federal law to be applied to all U.S. States unless a specific exception has been proposed by the state and granted by the USEPA. Primary standards have a Maximum Contaminant Level Goal (MCLG) and an enforceable Maximum Contaminant Level (MCL). The MCLG is a precautionary non-enforceable goal defined as "the level at which no known or anticipated adverse effects on the health of persons occur and which allows an adequate margin of safety". The MCLGs take into account only public health, without considering the technological feasibility of the analysis and/or treatment. In cases where treatment, analytical detection, and/or economic feasibility preclude achieving the MCLG, the SDWA specifies that the enforceable MCL be set as close to the MCLG as feasible using the best available technology and other means (considering cost). The USEPA drinking water primary standards require regular testing to ensure compliance with the regulations. The USEPA also can issue 'Health Advisories' for chemicals for which no regulation exists and limited data on occurrence and/or health effects may limit regulation development. Since 'Health Advisory' levels are non-enforceable, there is generally no opportunity for public comment and there is no penalty from the USEPA for non-compliance.

The SDWA includes a rigorous process for considering emerging contaminants, called the Candidate Contaminant List (CCL) and Unregulated Contaminant Monitoring Rule (UCMR). The CCL process is prioritizing chemicals from a "universe" of potential contaminants which may require regulation for protection of drinking water. Chemicals are prioritized based on their occurrence and health metrics, including production volume, severity of health outcome, magnitude of national occurrence, and prevalence of occurrence (Anderson et al., 2010). There are limitations as the process occurs in five year cycles and can only list, ultimately, a finite number of substances of which only a few are actually considered for regulatory determinations. Another mechanism for evaluating emerging contaminants in U.S. drinking water is the UCMR, which requires monitoring of no more than 30 unregulated substances in essentially all major U.S. drinking water systems in 5-year cycles. The UCMR requires use of USEPA approved analytical methods and certified laboratories in order to better ensure comparability of the resulting data. Both, the CCL and UCMR are subjected to public comment, including multiple opportunities for nomination of chemicals, before being finalized and implemented for monitoring.

The Clean Water Act (CWA) operates independently from the Safe Drinking Water Act and is predicated on the protection of ambient waters of the USA. The CWA is the predominant regulation for wastewater discharges and thus plays an important role in potential mitigation of TOrC loading to the aquatic environment. The National Pollutant Discharge Elimination System (NPDES), which is established under the CWA, regulates discharge of pollutants into U.S. waterbodies from point sources. Most states are authorized by USEPA to issue NPDES permits for point source discharges. However, the CWA does not have a systematic process for chemical prioritization and historically has been more focused on nutrients, metals, and pathogens. Regardless, the CWA establishes ambient water quality criteria that are to be protective of human and environmental health. This sometimes leads to very stringent criteria for bioaccumulative substances since the protection also must apply to potential human consumption of aquatic organisms (mainly fish). Thus, the regulatory limits for some bioaccumulative substances are very strict in the CWA. For instance, the current ambient water quality criteria value for DDT is 0.001 µg/L (USEPA, 2017c). The mechanisms for the consideration of emerging contaminants for ambient water quality criteria development are unclear. The CWA also includes provisions for designating U.S. waters as ‘impaired’, which then requires establishment of total maximum daily loads (TMDLs) on a watershed level. The TMDL is an enforceable standard for the maximum amount of a substance that can be loaded to a water body in a single day, which accounts for both point source and non-point source contributions. Therefore, the substance responsible for the impairment of water quality has to be identified in a first step. After identifying compound sources, locations and loads, an assessment for the prioritization of relevant sources is conducted. Further factors, such as land usage, population and others, which can affect the load of a substance have to be considered, too. Load allocations to point and non-point sources and additional safety margins are used to estimate TMDLs (USEPA, 2011a). TMDLs are intended to restore unimpaired conditions in a waterbody and can be implemented with accompanying implementation plans, which allow an effective and comprehensive restoration of water quality. Ultimately, the USEPA has granted the majority of states the right to issue permits and enforce regulations by reporting those data to the USEPA on a regular basis. Similarly to the SDWA, states may apply more stringent and expansive requirements, but not less than the USEPA federal requirements. U.S. water standards are summarized in Table S- 1 on page 168.

4.3.2 European Union

4.3.2.1 Socio-political background

The EU consists of 28 member states (2016) with a total population of 507 million people (Eurostat, 2016). Population density varies from 466/km² in the Netherlands to 16/km² in Finland (bpb, 2011). Its land surface area of 4,324,782 km² covers a large diversity of landscapes, including permafrost areas, coastal areas, high alpine areas and Mediterranean zones. Drinking water is mainly abstracted from ground and surface water and approximately 250,000 million m³ of freshwater are annually utilized (Eurostat, 2017). Wastewater is treated in approximately 45,000 publicly-owned wastewater treatment plants across Europe (FAO, 2016; Eurostat, 2017).

4.3.2.2 Development of environmental regulations

EU water legislation was initiated in 1975 (at that time the European Economic Community) with water quality standards for rivers and lakes, which served as drinking water source. Although most member states already had implemented own national regulations at that time, quality targets for drinking water were established on an EU-wide basis and several directives for urban wastewater treatment, industrial emissions and other water and environment related topics were passed in the following decades. In 1995, the EU parliament's Environment Committee and the Council of Environmental Ministers requested the European Commission to establish a comprehensive water policy addressing emerging concerns regarding impaired water quality within Europe (European Commission, 2016). The resulting Water Framework Directive (WFD, 2000/60/EC) was implemented in 2000 (European Commission, 2000). Driven by the precautionary principle, the core objective of the WFD is to avoid long-term deterioration of water quantity and quality and to restore conditions of all water bodies close to their natural state without noticeable impact from human activities. The implementation of the WFD is intended to result in sustainable management of water resources on the basis of river basins, while taken into account social, environmental and economic aspects. In order to fulfill these objectives, other regulations were amended, leading to a portfolio of interrelated directives. The interactions among these individual pieces of legislation are designed to control substances that present a significant risk to or via the aquatic environment.

4.3.2.3 Existing approaches for regulated TOrCs

In the EU, chemicals which are likely to accumulate in sediments or organisms are assigned sediment or biota standards. These chemicals are summarized as priority substances (PS) under Annex X of the WFD and Directive 2013/39/EC (European Commission, 2013). Chemicals which are classified as persistent, toxic or bio-accumulating (persistent organic pollutant under Stockholm Convention, substance of very high concern under REACH, persistent, bio-accumulative and toxic (PBT criteria) under Regulation EEC No. 793/93)) are defined as priority hazardous substances (PHS). For these chemicals, member states have to implement measures (in accordance with WFD) which shall result in a progressive reduction of pollution by priority substances and phasing out of priority hazardous substances emissions into the environment.

For chemicals which have been identified to pose a risk to the environment, environmental quality standards (EQS) are proposed (European Commission, 2013). EQS are derived on the basis of toxicological evidence with a base set of organisms from three trophic levels (i.e., fish, daphnia/crustaceans, algae/macrophytes). These studies are intended to cover long- and short-term effects resulting from chemical exposure (European Commission, 2011). Based on the used set of data appropriate safety or assessment factors (10 to 10,000) are applied. Annual average concentrations for environmental quality standards (AA-EQS, based on chronic toxicity data) and maximum acceptable concentrations for environmental quality standards (MAC-EQS, based on acute toxicity data) are determined for both fresh and salt water. EU EQS-values for organic chemicals are summarized in Table S- 2 on page 169.

For ground and drinking water pesticide concentrations, blanket values which are not toxicologically derived are applied in the EU. Single pesticide concentrations are not allowed to exceed 0.1 µg/L in water bodies and the concentration (sum) of all pesticides has to be lower than 0.5 µg/L (Council of the European Union, 1998; European Commission, 2006b). If stricter water quality standards exist for certain pesticides, these apply. Regulation EC 1107/2009 provides the union-wide basis for the registration and utilization of pesticides (European Commission, 2009a). Permissions for the utilization of pesticides are granted on the level of member states for ten years. After this period, permissions have to be requested again and based on the current state of science a new assessment is conducted. The regulation allows a zonal regulation of pesticides in southern, central and northern Europe. If the regulation of pesticide is requested in more than on country of a zone than these conduct a joint assessment, but each member state decides regarding the regulation independently considering national and ecological conditions. Compounds with toxic effects classified by GHS-category 1A or 1B (cancerogenic, mutagenic, or reproduction toxicity) will not be authorized in the EU. The directive 2009/128/EC provides a framework for the sustainable use of pesticides (European Commission, 2009b). It requires member states to implement national strategies for reduction of pesticide usage and to promote alternative agricultural techniques, which are less dependent on pesticides. Pharmaceuticals are authorized through the European Medicines Agency (EMA). The registration process includes a careful assessment of risks and benefits also considering ecological aspects (Hart, 2005).

All aspects concerning registration, evaluation, authorization and the restriction of chemicals (REACH) within the EU are combined under one regulation, which came into effect in 2007 (European Commission, 2006a, 2010). The European Chemical Agency (ECHA) was established in Helsinki (Finland) to oversee the REACH program. Following the "no data – no market" principle, producers and importers of chemicals are required to register all compounds at ECHA. Subsequently, provided data is evaluated and (partly) checked for conformity with REACH requirements. Through authorization and regulation of chemicals, the use of chemicals with intolerable risks for health and/or environment can be restricted or banned. Regulation can even be applied to chemicals which have already been placed on the market, when new data suggest significant concern regarding the safety of a compound. Member states are involved in the evaluation process of documents which have to be submitted under REACH. This provides member states the opportunity to influence the registration of compounds.

4.3.2.4 Water quality protection from TOrCs and consideration of new candidates for regulations

In the EU, water quality is monitored, assessed and controlled on the basis of (transnational) river basins for which management plans have to be established and implemented (Kallis, 2001). Member states are responsible for the designation of river basins and for specifying authorities for compliance monitoring. These programs provide a comprehensive characterization of the river basin, details about monitoring and the set of measures which have to be implemented to improve water quality. Progress on the implementation of river basin management plans is reviewed every six years. Water quality is determined by assessing the ecological and chemical status for each river basin (Figure 4-1). The classification scheme of waterbodies distinguishes a high, good or moderate status. The biological status includes the composition and abundance of aquatic flora and fauna and supporting physico-chemical parameters like temperature, salinity, nutrient conditions, and others. Good biological status is achieved when waterbodies show "*only low levels of distortion resulting from human activity*" (European Commission, 2000). The chemical status is defined by the presence/absence of priority hazardous compounds and other compounds which enter the aquatic environment in "*significant quantities*" (European Commission, 2000). Good chemical status is achieved when no EQS is exceeded. The responsibility to achieve a 'good chemical and ecological status' of water bodies and to implement basic and supplementary measures is directed to individual member states, which can further delegate these duties to state agencies or federal authorities.

Compounds, besides PS and PHS, which enter the aquatic environment in significant quantities and are frequently detected in water bodies can be assigned as river basin specific pollutants (RBSPs) and included in routine monitoring in the river basin. RBSPs can be reported to the European Commission and if environmental relevance is stated, be added to a watch list for additional investigations (European Commission, 2015a). These additional compounds have to be monitored in all water bodies of the EU (Table S- 3 on page 171). Ultimately, watch list compounds can become PS or PHS if environmental relevance and EU-wide occurrence has been confirmed. Risks posed by mixtures of different TOrCs have high relevance for the protection of water quality, but current EU regulations do not include comprehensive tools for the management of compound mixtures. Therefore, intensive studies were conducted to investigate the options to include strategies to manage risks from compound mixtures into the existing regulative framework (Kienzler et al., 2014; Evans et al., 2016).

Basic measures required to achieve the environmental goals of the WFD and compliance with other EU directives are primarily regulatory and administrative instruments, which are often not sufficient to reach the defined quality goals, such as the reduction of pollution from agricultural sources (European Commission, 2014). Supplementary measures are a set of diverse measures, which might all contribute to the achievement of environmental goals, supporting already implemented basic measures. Member states can choose appropriate measures from a catalogue of measures and design suitable strategies for individual river basins. Cost-effectiveness of measures, cost recovery and incentive water pricing and other economic requirements under WFD have to be considered in the selection of these measures.

Although, the establishment of operational measures for the improvement of environmental status was due in December 2012, only 23% of the WFD-specific basic measures were reported to be established at the EU level at the end of 2014, while 66% were ongoing and 11% not yet initiated (European Commission, 2015b). The implementation of supplementary measures was completed by 29% (54% ongoing, 17% not initiated yet). The WFD is under regular revision, aiming to update strategies on a regular basis and being able to respond to upcoming challenges. For the next revision in 2019 several improvements, including improved risk assessment for mixtures, the introduction of effect directed analysis for the identification of unknown compounds and passive sampling among others have been proposed (Brack et al., 2017).

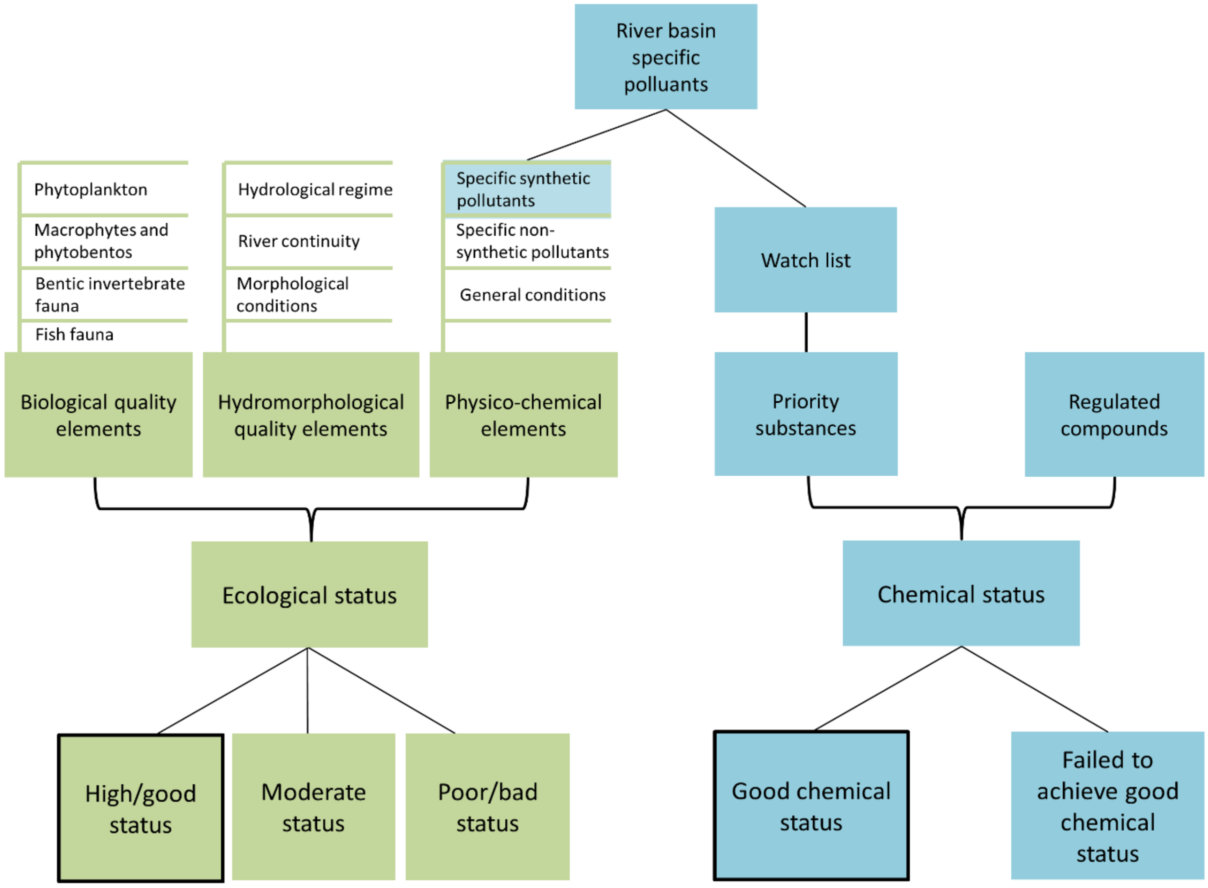

Figure 4-1: Framework to assess a "good status" of surface waters under the EU Water Framework Directive.

4.3.3 Germany

4.3.3.1 Socio-political background

The Federal Republic of Germany, as one of the largest countries within the European Union, consists of 16 federal states, comparable to the U.S., but with less autonomy of the individual states. As a member state of the EU, Germany is legally bound to implement regulations and directives of the EU, but national implementations can be stricter than the legal requirements of the EU. Approximately 61% of the drinking water in Germany is abstracted from groundwater, other sources are surface water (river and lakes), spring water, bank filtrate and artificially recharged groundwater (Statistisches Bundesamt, 2013). Drinking water is provided by 14,983 water treatment plants and municipal wastewater is treated in 9,307 publicly-owned wastewater treatment plants across Germany (Statistisches Bundesamt, 2015a, 2015b).

4.3.3.2 Development of environmental regulations and existing approaches for regulated TOrCs and new candidates for regulations

German water policy is based on the Water Resources Act (WHG), which was established in 1960. By 2011, the EU WFD and other directives were implemented in German law (Bundesregierung, 2009; 2011). The Water Resources Act and the Surface Water Directive (OGewV) provide a framework for all federal states, which are responsible to enforce water quality goals and the implementation of measures to mitigate negative impacts on water bodies.

For managing TOrCs in the aquatic environment, Germany uses a combination of toxicity-based enforceable water quality standards and non-enforceable precautionary principle based health advisory levels. By implementing the requirements of the EU WFD, compliance with environmental quality standards for PS and PHS has to be achieved in water bodies. In addition, the German Surface Water Act lists 162 potential river basin specific pollutants (i.e. organic and inorganic compounds) and (enforceable) quality standards (Bundesregierung, 2011). These have to be included in water quality monitoring programs if the occurrence of these compounds is likely and environmental concentrations are higher than 50% of the water quality standard. These compounds can be reported to the European Commission for consideration of future regulation.

4.3.3.3 Other regulatory programs targeting TOrCs

To achieve compliance with WFD water quality goals, five of the sixteen federal states in Germany have initiated specific programs to evaluate the potential of source-control measures and advanced wastewater treatment processes to reduce the discharge of TOrC to receiving water bodies. Regarding treatment approaches, the state of Baden-Wuerttemberg currently exclusively focuses on investigations of adsorptive removal of TOrC in wastewater treatment plants by powdered activated carbon (PAC). This preference is motivated by the state’s track record in applying activated carbon for the treatment of wastewater from textile industry, where activated carbon has been traditionally utilized for discoloring of wastewater. Initial large-scale treatment plant upgrades were conducted at select wastewater treatment facilities representing a combined treatment capacity of approximately 10% of the total wastewater flow generated in the state. An expansion to include additional facilities, doubling the amount of treated wastewater to 20%, is targeted to be completed until 2022 (Umweltministerium Baden-Wuerttemberg, 2012). In the city-state of Berlin, drinking water is mainly abstracted from riverbank filtration and groundwater. The rivers where riverbank filtration is practiced also receive discharge from the city’s WWTPs. Due to this *de facto* potable water reuse situation, the occurrence of persistent TOrC in surface water supplies is a major concern for Berlin’s drinking water supply. To reduce the discharge of TOrC into the aquatic environment, activated carbon filtration and ozonation for advanced water treatment has been investigated and one of the large-scale WWTP is scheduled to be upgraded with ozonation by 2021 (Huckele and Track, 2013). The state of North-Rhine Westphalia has investigated a broad range of technologies, including adsorptive technologies (activated carbon) (Grünenbaum, 2011) and ozonation (Neumann et al., 2011) as reduction measures for TOrC. In addition, membrane technologies (Pinnekamp and Wessling, 2012) and advanced oxidation processes (Pinnekamp, 2012) have been considered. As a highly industrialized and densely urbanized area with many small rivers receiving discharged wastewater having little natural dilution, North-Rhine Westphalia is faced with a large number of river districts that exhibit a poor ecological and chemicals status. As of February 2016, 14 of a total of 381 WWTPs (with design capacities of more than 10,000 people equivalents) have been upgraded with advanced wastewater treatment processes including ozonation or activated carbon adsorption (Kompetenzzentrum Mikroschadstoffe North-Rhine Westphalia, 2016). For 12 additional WWTPs, pilot- and demonstration-scale studies were conducted and 95 facilities were subject to feasibility studies. The state of Bavaria, the largest and second most populated German federal state, has initially established a river basin TOrC load model for the state's rivers and streams. Based on this effort, only a few river sections were identified where EQS of priority and priority hazardous substances and river basin specific pollutants are exceeded. The state has launched a research program to investigate advanced treatment options including ozonation with subsequent biological granular activated carbon filtration as well as advanced oxidation processes (UV/hydrogen peroxide) as potential mitigation strategies for WWTPs discharging to streams where EQS are exceeded. Additional federal states, like the state of Hesse, have proposed to implement advanced wastewater treatment technologies to reduce TOrC emissions from selected WWTPs.

The efficiency of implemented measures by specific treatment processes is most commonly assessed by monitoring the removal of selected indicator compounds during wastewater treatment processes rather than confirming desired reductions of (eco-)toxicological impacts on the receiving streams. The key quality criterion in the aquatic environment is compliance with the EU EQS. Although the EU Water Framework Directive aims to prevent the exposure of compounds to aquatic life, most efforts undertaken in EU member states so far are based on emission reduction limiting the discharge of compounds to the aquatic environment.

While advanced wastewater treatment technologies were evaluated through grant programs provided by individual federal states, as of today there are little if any synergistic effects in these efforts among the various states and collaborations between states do hardly exist. Some states have formalized research efforts and established institutions that are charged to coordinate assessment studies and the implementation of advanced treatment processes. Examples are the 'Competence Center for Trace Organic Compounds' in Baden-Württemberg (KomS-BW) or the North-Rhine Westphalia 'Competence

Center Micropollutants'. The mediation of research and results of feasibility studies between federal states could increase efficiency of implementation efforts and lower cost for all federal states. A coordinating institution, surveilling research efforts and implementations at the national level does not exist. In 2016, the German Ministry of the Environment, Nature Conservation, Building and Nuclear Safety initiated a stakeholder process to agree on a coordinated national TOrC strategy, which is addressing options for compounds substitutions where feasible, source control measures, and upgrades of wastewater treatment facilities (Hillenbrand et al., 2016; BMUB, 2017).

4.3.4 Switzerland

4.3.4.1 Socio-political background

Switzerland is located in the Alps, in the center of Europe, and is comprised of 26 cantons. Geographies range from low land areas to high alpine regions. Drinking water in Switzerland is supplied via springs (40%), groundwater (40%), and surface water (20%, mainly lakes) (SVGW, 2014). The 759 publicly-owned Swiss wastewater treatment plants represent a total wastewater capacity of 10.4 million population equivalents (Maurer and Herlyn, 2006).

4.3.4.2 Development of environmental regulations

The first Swiss water protection act came into force in 1957. The revised version of 1991 aims to provide a comprehensive protection for all water bodies. The latest amendment was adopted in January 2016 and includes one of the most comprehensive management strategies for trace organic compounds in water bodies worldwide. This amendment was the result of a process that included investigations on the risks for environmental health posed by TOrCs. A national research program on effects of endocrine disrupting substances in water bodies on environmental and human health (2002 – 2007) provided first evidence that wastewater treatment plant effluents are a main source for TOrC in Swiss surface waters (NRP 50, 2008). Follow up studies investigated TOrC in wastewater effluents, quantified discharged loads to receiving streams and evaluated potential removal techniques for TOrCs at wastewater treatment plants (BAFU, 2009; Gälli et al., 2009; Ort et al., 2009; Abegglen and Siegrist, 2012). Public awareness and concerns about potential (environmental) health risks posed by TOrC resulted in an initiative that the Swiss government started to address TOrC in federal legislation. This initiative aimed to reduce discharge of TOrC to receiving streams in general by 50% for the entire country. Therefore, the Swiss Environmental Agency was commissioned to develop a national strategy for the management of TOrC in water bodies. As a result, a catalogue of several different measures was developed. These measures include regulatory initiatives (i.e., compound regulation, substitution, etc.), management approaches (i.e., consolidation of smaller WWTP service areas to be connected to larger facilities), decentralized source control approaches, and the implementation of advanced wastewater treatment processes (Gälli et al., 2009).

4.3.4.3 Existing approaches for regulated TOrCs

Switzerland is not a member state of the EU and thus not implementing EU legislation. In some areas, Swiss legislation is partly harmonized with EU standards, like the regulation and authorization of chemicals, which is geared towards EU REACH requirements. Strategies for the management of TOrCs are independent from EU policy. The 2016 amendment to the Swiss water protection bill specifies discharge limits for TOrCs in wastewater depending on the capacity of a WWTP (Swiss Federal Council, 2017). Blanket values are applied for compound groups, such as pesticides or hydrocarbon compounds in environmental waterbodies and drinking water, comparable to the EU approach (EDI, 2017; Swiss Federal Council, 2017). For drinking water, threshold values for toxicologically assessed compounds are assigned. In addition, not fully assessed TOrCs with known chemical structure should not exceed 0.1 µg/L when a genotoxic potential is assumed and not exceed 10 µg/L for toxic compounds (Cramer classes I to III) and organophosphate (EDI, 2017). Pesticides are regulated in Switzerland by the Federal Office for Agriculture, which provides an assessment of human and/or environmental health risks (Swiss Federal Council, 2017).

4.3.4.4 Water quality protection from TOrCs and consideration of new candidates for regulations

The applicability of advanced wastewater treatment processes for the reduction of TOrC from point sources such as wastewater was investigated and two technologies, namely ozonation and powdered activated carbon, showed the best applicability and compound removal characteristics for a shortlist of indicator chemicals in numerous pilot- and full-scale studies for the conditions in Switzerland. Both technologies were able to achieve an overall removal of at least 80% of these specified TOrC indicators (Table 2). Other technologies, like advanced oxidation processes (AOP) or membrane technologies were considered as well, but not found to be suitable at the time. However, investigating alternative treatment processes is encouraged by the overall program. The subsequent legislative process involving the public and all stakeholders resulted in a broadly supported adaption of the Swiss Water Protection Act in 2014, which came into effect in January 2016 (Federal Assembly of the Swiss Confederation, 2016). This legislation requires the nationwide implementation of advanced wastewater treatment processes in WWTPs serving more than 80,000 people equivalents, plants discharging to surface water used for the abstraction of drinking water, and WWTPs contributing more than 10% of the total flow to a receiving stream (Eggen et al., 2014). This upgrading program will include approximately 100 of the total of 759 Swiss wastewater treatment plants by 2040. The program is funded by a national fund (75% of total costs) and fees paid by wastewater producers (polluter-pays principle) (BAFU, 2012).

After full implementation of this program, approximately 50% of the total volume of wastewater generated in Switzerland will be subject to advanced treatment each achieving a removal of at least 80% of selected TOrC indicators (Eggen et al., 2014; Swiss Federal Council, 2016). Twelve indicator compounds were chosen on the basis of ubiquitous occurrence in surface or wastewater, insufficient removal by biological wastewater treatment (<50%), and moderate to good removal by the two favored advanced treatment processes (>60%) (Götz et al., 2015) (Table S- 4 on page 172). TOrCs which are widely detected in Swiss waterbodies, occasionally detected at high concentrations or representing a high specific toxicity are summarized in Table S- 5 on page 173 (BAFU, 2015). Treatment efficiency is assessed by monitoring the removal of at least 6 indicator compounds from the proposed list in wastewater effluents. The success of TOrC discharge reduction on environmental health will be assessed by monitoring receiving stream impacts after mitigation measures have been implemented.

4.3.5 Australia

4.3.5.1 Socio-political background

Australia consist of the main Australian continent land, Tasmania and several islands, which are administratively organized in eight federal states and territories. The population is concentrated along the coastal areas, while the center of the Australian continent is sparsely populated. Drinking water is abstracted from ground and surface water by more than 80 water treatment plants, but in some locations also seawater desalination is used (Australian Government, 2017). Wastewater is treated in approximately 577 publicly-owned WWTPs (Australian Government, 2017).

4.3.5.2 Development of environmental regulations and existing approaches for regulated TOrCs

Australian Water Quality Guidelines for Fresh and Marine Waters were released in 1992 and are constantly reviewed since that time (ANZECC & ARMCANZ, 2000). The guidelines aim to provide a broad range of (non-enforceable) tools for the management of environmental water quality. To achieve a sufficient protection of water quality and environmental health, tools proposed by the guidelines have to be adjusted to local conditions. During the last years, many regions in Australia were challenged by extreme weather phenomena like widespread droughts or extreme flooding. The millennium drought from 2001 to 2009 led to a severe shortage in water supply (van Dijk et al., 2013). As a consequence, reuse of local impaired water sources such as greywater, stormwater or treated wastewater effluents were considered viable alternatives to reduce stress on drinking water supplies (Rathjen et al., 2003; SECITA, 2002; NWQMS Phase 1, 2006; Leusch et al., 2014). This resulted in regulatory action and particularly in the development of the Australian Guidelines for Water Recycling, addressing non-potable reuse applications (NWQMS Phase 1, 2006) and the augmentation of drinking water supplies (NWQMS Phase 2, 2008). At this time, the Australian Drinking Water Guidelines (ADWG) have defined measures for TOrC and pathogens for human health protection (NHMRC and NRMMC, 2016).

4.3.5.3 Existing Approaches for Regulated TOrCs

The Australian water quality guidelines for fresh and marine waters aim to provide a comprehensive protection of the environment. Therefore, the guidelines recommend to implement integrated approaches combining numerical values for chemical compounds, water quality monitoring, toxicity assessment and biological monitoring (ANZECC & ARMCANZ, 2000). Numerical (trigger) values are derived on the basis of toxicity data from several species. Depending on the quality of data, the reliability of trigger values is classified into high (derived from no observable effect concentrations), moderate (derived from short term acute toxicity tests) and low reliability (insufficient data set). To ensure the protection of a wider range of species, toxicity data are extrapolated. Based on the statistical distribution method, four different protective levels of trigger values are derived. The levels are expected to protect 99%, 95%, 90% or 80% of the species in an ecosystem. The decision, which protective level is implemented should be the result of a process, considering stakeholders, community and ecosystem conditions. Exceedance of implemented trigger values for TOrCs can result in the implementation of mitigation measures or further investigations regarding the risks for the ecosystem posed by the exceedance. This can include local (important) species, background concentrations, mixture interactions and others. As a result, new guideline values can be derived and if these are still exceeded, direct toxicity assessment should be conducted, deciding about the establishment of immediate actions (ANZECC & ARMCANZ, 2000).

The Australian drinking water guidelines follow a comparable strategy as the guidelines for fresh and marine waters (NHMRC and NRMMC, 2016). The management of drinking water quality is based on 12 elements, combining water quality requirements, stakeholder interests, system analysis and management elements, research and development, community involvement, operator training, and long-term evaluations of results. The framework includes elements of the Hazard Analysis Critical Control Point (HACCP) approach and the international standard ISO 9001 specifying requirements for a quality management system.

The Australian management strategies for drinking water quality represent a three-stage risk management framework. The drinking water guidelines primarily aim to protect against health risks posed by pathogens. These are controlled by the establishment of multiple barriers. TOrCs groups, such as disinfection by-products (DBPs), pesticides, pharmaceuticals and endocrine disrupting compounds (EDCs) are considered in the guidelines. The implementation of measures to reduce DBP concentrations in drinking water are advised by the guidelines, but disinfection efficiency should not be decreased by these measures. For pesticides, acceptable concentrations based on acceptable daily intake (ADI) values are defined and monitored. Guideline values for pharmaceuticals and EDCs potentially present in recycled water are provided in the Australian national guidelines for water recycling (NWQMS Phase 2, 2008). These guidelines aim to provide guidance for the reuse of water for drinking water purposes. Comparable to the drinking water guidelines, a combination of risk assessment, management and monitoring is used to achieve a sufficient level of health protection. This approach can be implemented for a broad scale of applications from single households to complete watersheds. If a risk is identified, preventive measures are to be implemented as close as possible to the source of the hazard and criteria for monitoring the implementation success of these measures are to be defined (NWQMS Phase 2, 2008). For recycled water systems specifically, source control and treatment barriers are proposed (NWQMS Phase 2, 2008). Source control measures aim at preventing or minimizing pollution in sewage and storm water collection systems by industrial, human or animal wastes. Treatment barriers fall into different categories: Primary and secondary treatment (reducing the concentration of microbial pathogens, nutrients and bulk organic matter) and tertiary treatment (additional removal of microbial pathogens and organic chemicals), including detention in reservoirs and soil-aquifer treatment (NWQMS Phase 2, 2008).

Prevention of all health risks is infeasible, instead, an acceptable risk is scientifically defined and compliance towards this goal is monitored. For microbial risks benchmarking, the WHO's concept of disability adjusted life years (DALYs) has been adopted. The DALYs concept could not be expanded to the risk assessment of TOrCs in general, because of insufficient evidence and data on dose and exposure dependent (human) health effects (NWQMS Phase 2, 2008).

Chemical compounds produced in or imported into Australia have to be registered under the National Industrial Chemicals Notification and Assessment Scheme (NICNAS, 2016). The registration procedure includes a quantitative and qualitative risk assessment for the protection of human health and the environment. The annually produced and imported quantities of a chemical have to be reported to the registering authority.

4.3.5.4 Water quality protection from TOrCs and consideration of new candidates for regulations

Human health risks from TOrCs are primarily assessed considering existing guideline values. These values serve as basis for the implementation of enforceable water quality standards by federal states. If guideline values are not available in Australian or international guidelines or regulations, then toxicological information has to be collected and used for the definition of thresholds of toxicological concern (TTC) on the basis of no observed effect levels (NWQMS Phase 2, 2008). Specific compound properties (genotoxicity, etc.) result in the definition of more rigorous TTC values. Risks for the environment by TOrCs are assessed by comparing the measured environmental concentrations to the guideline values for hazards related to the most sensitive environmental endpoint. These guideline values are based on the TOrC concentrations protective of 80 to 99 % of all species (NWQMS Phase 1, 2006) (Table S- 6 on page 175). For pharmaceuticals an alternative concept for the derivation of guideline values is applied. While pharmaceuticals for veterinary use are assessed using the ADI concept, guideline values for pharmaceuticals which are administered to humans are derived on the basis of therapeutic dosages. The lowest therapeutic dose of a pharmaceutical is divided by a safety factor of 1,000 to 10,000. This is expected to provide a sufficient degree of safety from adverse effects posed by pharmaceutically-active compounds. Metabolites of pharmaceuticals are assessed following the same concept.

4.4 International comparison of the management approaches

While the management strategies of the investigated countries target similar categories of TOrC, the analysis of individual management approaches conducted in this study revealed that underlying principles differ and there is not a single approach which is consistently applied ubiquitously. A summary of investigated regulations and assessed criteria is provided in Table 4-2. The USA is currently mainly focusing on the management of TOrC by defining specific water quality standards for individual chemicals. Compound discharges into impaired waterbodies (TMDLs) are currently mainly regulated by implementing limits for pathogens, heavy metals and nutrients. A comprehensive implementation of TMDLs for TOrCs has not been initiated, yet. Australia has implemented an integrated risk management approach for TOrCs in environmental waters, drinking water and potable water reuse and therefore is mainly focusing on addressing the risk to human health. The EU has implemented a comprehensive legislative framework, mainly focusing on managing risk to the aquatic environment. Germany as an EU member state is in the early stage of translating EU directives and requirements into management strategies for TOrCs, so far with an inconsistent national approach. In 2016, Switzerland launched a progressive and comprehensive regulatory action for the reduction of TOrC emissions into water bodies. In a nationwide program wastewater treatment facilities are being upgraded over the next 25 years with advanced treatment processes targeting blanket 80% removal of selected TOrCs.

The following sections provide a focused analysis of similarities and differences between the underlying principles of these international regulatory approaches regarding the initially introduced three evaluation criteria, namely:

1. The extent to which strategies manage different groups of TOrCs;
2. Implementation and compliance strategies specific to the management of human and aquatic health risk; and
3. The ability to adapt the implementation of management strategies to different site-specific watershed conditions.

Table 4-2: Overview of key criteria of international regulations considering TOrCs

Country/Region	**Considered regulations**	**Enforceability**	**Considered primary health endpoint**	**Toxicity-based elements**	**Emission avoidance-based elements**
United States of America	Clean Water Act	Yes	Aquatic life	Yes	No
	Safe Drinking Water Act	Yes	Human health	Yes	No
European Union	Water Framework Directive	Yes	Environmental health	Yes	Yes
	Drinking Water Directive	Yes	Human health	Yes	Yes
Switzerland	Water Protection Act	Yes	Environmental health	No	Yes
	Drinking Water Act	Yes	Human health	No	Yes
Australia	Water Quality Guidelines for Fresh and Marine Water	No	Environmental health	Yes	No
	Drinking Water Guidelines	No	Human health	Yes	No
	Guidelines for Water Recycling	No	Human health	Yes	No

4.4.1 Approaches for managing the different groups of TOrCs

With currently several ten thousands of chemical compounds in regular use and the continuous improvement of environmental chemical analysis, the number of detectable compounds in environmental water samples is constantly increasing (Schwarzenbach et al., 2006). In addition, it is common knowledge that many parent compounds form transformation products either during water treatment or after release into the aquatic environment. This occurrence pattern results in a complex universe of chemical compounds in impaired waterbodies. A particular challenge for drinking water supplies is the detection and identification of highly polar and persistent TOrCs (Reemtsma et al., 2016). These compounds can hardly be detected and monitored by currently uses state-of-the art analytical techniques as reversed phase liquid chromatography coupled to high resolution mass spectrometry. Therefore, alternative techniques with significantly broader polarity spectrum of detectable compounds are needed and have recently been proposed for the screening of TOrCs

(Bieber et al., 2017). To identify environmentally or health relevant TOrCs, prioritization approaches are required. These can be based on compound production volumes, occurrence data in waterbodies, toxicological assessments and other compound specific parameters. Such prioritization schemes are implemented in the USA through the CCL process under the SDWA or the priority substances and watch-list selection process within the EU WFD. The well-structured CCL process provides a very comprehensive approach to select emerging constituents from the universe of chemicals. While the CCL process is currently applied to compounds in drinking water sources, there is no similar structured process for chemical prioritization for ambient water qualities in the USA. Adopting the concepts of the CCL process to water bodies is possible, but regional and seasonal variations in TOrC occurrences need to be considered. In addition, current limitations of the CCL process, such as the five year cycles and the strong focus on compound occurrence over health risk concerns should be reconsidered. To address regional variability, the approach of defining river basins like in the EU might be more appropriate to identify locally relevant compounds to human health and the environment. Inspired by the U.S. CWA, the EU WFD covers EU-wide detected compounds by setting union-wide enforceable environmental quality standards considering short- and long-term effects to aquatic life (Hering et al., 2010). In addition, member states can define additional river basin specific pollutants. This offers an opportunity to address regional specific compound occurrence, which is an important aspect, when aiming to adjust management strategies to local situations. As a consequence, there is a constant challenge to integrate newly detected TOrCs into implemented management strategies.

Another option to capture the universe of chemicals, is to establish a comprehensive framework of regulations addressing the entire life cycle of chemicals including chemical use, production, distribution, and disposal. This allows managing hazards from TOrCs in all stages of their life cycle and to identify and control potential emission pathways including drinking water supplies early on in the process. However, such an approach requires the implementation of a superior management strategy resulting in interrelated regulations. The EU is currently establishing such a comprehensive framework linking chemical regulation, production, usage and disposal with environmental and health protection (Dudutytė and Kislenko, 2011). The implementation of such interrelated frameworks is very challenging, because many political and industrial stakeholder interests have to be balanced and existing regulation have to be synchronized. Ultimately, proper coordination between different regulations, programs and efforts is key to a successful implementation of mitigation strategies. This is especially important when responsibilities are delegated to subordinated governmental levels in member countries and regional governments. Here centralized institutions, who are in charge with coordinating and evaluating the implementation progress of measures at the national level are key to success. Targeted coordination at the national level has been proven effective in Switzerland, where the Federal Office for the Environment coordinates evaluation and implementation of mitigation measures for TOrCs. In comparison, in the EU each member country is currently responsible for the implementation of control measures within its territory but approaches, expertise, and lessons learned are rarely shared and coordinated among the member states, resulting in redundant efforts and in parts ineffective use of available resources. Not surprisingly, economically stronger member countries in the EU are generally in a better position to implement the WFD directives more effectively than economically weaker countries given the multiple objectives in implementing water policy measures (European Commission, 2015b). Similar differences can be observed at the state level of individual member countries, for instance in Germany. Here, five of sixteen federal states (generally those with higher population density) have to date initiated programs to manage TOrC in water bodies. The German Federal Ministry for the Environment, Nature Conservation, Building, and Nuclear Safety just recently has started to initiate steps to coordinate these efforts better at the national level (BMUB, 2017). The implementation of responsible agencies in member states and a well-orchestrated exchange of results and experiences between states through an overseeing agency level would help to take advantage of synergistic effects and allow states with less financial resources to implement required measures more easily.

4.4.2 Implementation strategies to manage human and aquatic life risk

The overall objective of management strategies is to reduce (potential) risks for humans and/or aquatic life posed by the presence of TOrCs in waterbodies. The management strategies of countries investigated in this study can be categorized in toxicity-based and/or emission avoidance-based management strategies. The core principle of toxicity-based management approaches is the determination and application of compound specific toxicity-based threshold concentrations, in order to assess the human and/or environmental health risk posed by a compound. In contrast, emission avoidance-based strategies aim to minimize the release of compounds into the aquatic environment without the need to determine specific quantitative, toxicologically justified standards. Based on the precautionary principle, these strategies follow the philosophy that organic chemicals originating from anthropogenic activities do not belong into natural water bodies or drinking water sources, thus their discharge should be either prevented or at least reduced.

4.4.2.1 Toxicity-based strategies

Toxicity-based strategies are derived following a quantitative risk management approach based on available toxicity data and by applying appropriate safety or uncertainty factors (NWQMS Phase 2, 2008; European Commission, 2011). In practice, such values are used as environmental and human health quality standards or maximum allowed concentration values for different types of sources and endpoints. Both, environmental and human health are the primary concern for surface water bodies used for fishing or as a source of drinking water supply. Human health protection is usually the main concern for groundwater or sensitive surface water bodies directly used for drinking water supply. However, the application of toxicity-based approaches is limited to already known compounds. Potential adverse effects caused by mixtures of different compounds and their transformation products are commonly not considered in these approaches (Faust et al., 2001; Escher and Fenner, 2011). Even when countries develop aquatic health standards for the same contaminant, the resulting limits might not be the same due to the use of different references in deriving threshold values. Here a better harmonization between countries would be helpful. The Organization for Economic Co-operation and Development (OECD) has established comprehensive guidelines for the testing of chemicals, which have been adopted by many countries (OECD, 2016). Although, countries might apply more or less stringent safety factors when deriving water quality standards, the same toxicological data basis could be used among countries.

4.4.2.2 Emission avoidance-based strategies

Following the paradigm of emission avoidance-based strategies, countries have developed different TOrC management measures. For example, in order to minimize the discharge of TOrCs to water bodies, pursuing generic quality goals can represent protective, precautionary concentration values. Such quality goals can be defined for compound groups. These values are not based on toxicity data of individual compounds but generally set at levels that are lower than typically known toxicity based quality standards. Switzerland and the EU have both adopted this concept when implementing water quality goals for pesticides (i.e., not to exceed 0.5 μg/L in total or 0.1 μg/L for individual pesticides). A similar approach exists in the USA by defining secondary drinking water standards, health advisories, or maximum contaminant limit goals (MCLGs). Following the same logic, the European Association of Waterworks has proposed a maximum total concentration of 0.1 µg/L per substance for all anthropogenic organic compounds in surface waters if no stricter health based threshold values exist, which are used for the abstraction of drinking water along all major European rivers regardless of whether the contributing compounds are health relevant (IAWR et al., 2013). This proposal extends the concept that the EU is currently applying to pesticides to all TOrC. With this approach the water utilities aim to reduce impacts on drinking water treatment and quality, but it would also provide benefits to water bodies by lowering compound releases into the aquatic environment. However, considering that most TOrCs occur at levels lower than 100 ng/L in receiving streams and that for some TOrCs there is evidence of adverse effects to aquatic organisms (i.e. endocrine disruption) and human health (e.g. elevated cancer risk due to N-nitrosamines) at the sub-nanogram per liter level, the actual environmental benefit of this concept remains uncertain (Kidd et al., 2007; Caldwell et al., 2008;

Krasner et al., 2013). The approach of setting (to a certain extent) arbitrary quality goals to restrict the release of TOrC requires acceptance by all stakeholders, which might be difficult to get. However, while this approach might not be ideal, it has the advantage that the regulatory process is not held up by the fact that toxicity data may not yet be available for known or unknown chemicals of concern.

4.4.2.3 Combined management strategies

Considering the individual national management strategies evaluated in this study, the regulatory approaches and programs in Australia, the EU, and the USA are comprised of both toxicity-based and emission avoidance-based elements to manage release of TOrCs into the aquatic environment and drinking water supplies (Table 2). However, toxicity-based strategies for management of TOrC are more commonly applied. Potentially hazardous compounds are identified, assessed through toxicological studies, and where considered appropriate subsequently regulated. The EU implemented a combined approach of toxicity and emission avoidance-based elements using quality standards for receiving water bodies and emission limiting values. Known hazardous compounds are regulated by environmental quality standards based on toxicity assessments, and the goal of 'good water quality' is supposed to be reached by emission reduction of TOrC following the precautionary principle. While the strategy of the EU primarily focuses on compliance with EQS for select compounds with proven adverse effects on the aquatic flora and/or fauna or human health, Switzerland pursues the goal to reduce the emission of all TOrC of anthropogenic origin from municipal wastewater irrespective of proven health concern. Switzerland favors a blanket reduction of chemicals and is pursuing an emission avoidance-based management strategy, aiming to reduce the load of TOrC to surface waters in general by implementing two advanced treatment processes in 100 out of approximately 700 municipal WWTPs. However, Switzerland did not define specific environmental quality standards for these chemicals, but is targeting a general load reduction by 50% nationwide. It is noteworthy, however, that only those chemicals will efficiently be removed which are amendable to either ozonation or adsorption onto activated carbon. These treatment processes are not able to remove highly polar chemicals that are not amendable to oxidation (e.g., chlorinated flame retardants; some artificial sweeteners) or adsorption (e.g., gabapentin).

Both, the toxicity-based and emission avoidance-based management strategies can be applied effectively to reduce risk to human and/or environmental health posed by TOrC depending on the toxicological endpoint to be protected. For example, the customized management strategy of the EU combines toxicity-based maximum allowed concentration values for hazardous compounds and a precautionary principle based compound emission prevention goal. The U.S., the EU, and Switzerland regulatory systems allow for the implementation of source control measures, discharge limits, and water treatment technologies for TOrC load reduction to the aquatic environment and/or prevention of drinking water contamination. The applicability and success of individual, customized measures is depending on the desired compound/compound groups and has to be assessed carefully.

Toxicity-based strategies are not feasible to apply to all TOrC detectable by contemporary analytical methods due to the required effort to assess the toxicological potential of all TOrC individually. Emission avoidance-based strategies are impossible to implement if they target a complete prevention of TOrC emissions by all sources and entry pathways. For point sources tailored individual advanced treatment technologies could be implemented in theory, but questions of feasibility, cost, and regulatory oversight remain. The emission prevention from non-point sources is even more difficult and often only enforceable by banning individual chemicals altogether from production and consumption. Measures like regulation or use restriction of chemicals, chemical substitutions, or take back programs can be motivated by both toxicity-based and emission avoidance-based strategies. Such measures might be very effective for the reduction of emission, but impact is strongly depending on the compound targeted. In case of regulation, use restriction and chemical substitution involve a lengthy political process and require the involvement of many stakeholders with uncertain outcome. For certain compound classes, such as pharmaceuticals, use restrictions are not applicable, due to compromised health care benefits. In case of chemical substitution, it has to be ensured that the substitute is as effective as the original substance and poses a significantly lower risk to human and environmental health. Take-back programs might be effective for specific compounds such as pharmaceuticals, but such measures require the establishment of suitable logistics and can only contribute to a reduction of emissions over a longer implementation period.

Toxicity-based approaches for individual chemicals have shown to be viable to estimate and control risks posed by single compounds to environmental and/or human health, but do not capture the risk from unknown substances such as transformation products and other not yet identified contaminants. Precautionary principle-based management strategies (i.e., emission prevention) instead provide the opportunity to address uncertainties associated with the fate of for example transformation products of TOrCs (Bedoux, et al., 2012). Ecological quality goals, as proposed in the EU aiming to restore water bodies to a natural status representing a state free of anthropogenic influence are ambitious considering the high degree of urbanization in many parts of Europe and global atmospheric transport and deposition of persistent compounds (Konstantinou et al., 2006; Vonberg et al., 2014). However, pursuing this goal in general will likely result in a constant improvement of water quality. Adverse effects from mixture toxicity (additive or potentially synergistic) are currently being discussed, but are not specifically addressed through any program of the targeted countries. The USEPA requirement of Whole Effluent Toxicity (WET) testing using fish and invertebrate species in both acute and chronic exposures would account to assess general toxicity from mixtures in wastewater outfalls; however, the endpoints considered do not include impacts such as endocrine disruption or endocrine behavior (USEPA, 2014c).

Assessing the efficiency of certain measures is critical during implementation. As for point source treatment upgrades such as conventional WWTPs, adsorptive (activated carbon) or oxidative (ozone) treatment processes are currently favored in Europe for TOrC load reduction into surface waters. High-pressure membrane filtration is also used in some cases. Process efficacy to remove TOrC by these processes is frequently assessed by a limited list of indicator chemicals. These performance indicators are primarily selected to assess process efficacy rather than representing ecotoxicological health relevance. For instance, in Switzerland 12 performance indicator chemicals were selected to assess the relative removal efficiencies of the two favored processes (i.e., activated carbon adsorption and ozonation). It is important to note that any process, due to its underlying removal mechanism, is limited to mitigate only certain classes of compounds as a function of their physicochemical or biological properties. Thus, following this approach in implementing and assessing a single treatment process is no guarantee to also removing chemicals with less favorable properties, which might also be toxicologically relevant. The use of a broad range of bioassays covering different toxicologically relevant endpoints for wastewater effluents might provide more specific information on the efficiency of wastewater treatment techniques as a management strategy to reducing adverse effects for the receiving stream than just monitoring a rather short list of indicator compounds. In addition, the use of bioassays also offers the benefit of considering the full spectrum of compounds present in a water sample and not only those which are detectable, although positive results of bioassays might not be fully transferable to human or aquatic health risks.

Following the logic of providing barriers against a larger number of chemicals, combining processes with different removal principles represent a more robust treatment approach capable of removing a wider spectrum of chemicals through multiple barriers as proposed in the Australian Water Recycling Guidelines. The combination of ozonation and activated carbon filtration or biologically-active filters is currently investigated by some countries in Europe. Such advanced treatment processes will lead to increased investment and operational costs. In Switzerland and the EU, the costs for advanced wastewater treatment are currently financed by federal and state subsidies as well as the rate payers or wastewater generators following the polluter-pays principle, resulting in slightly higher rates for wastewater disposal. In Switzerland, the national upgrade initiative is based on a broad public support and the slightly increased annual fees for advanced wastewater treatment (i.e. approximately $12 per capita and year) are accepted by the general public. In countries with lower economic strength, additional fees on wastewater treatment may not be acceptable, in particular until enforceable standards are not yet established at the national level.

4.4.3 Adapting strategies to site-specific conditions

Regional and local conditions have to be addressed during the implementation process of management strategies and ideally should be considered early on in the design of a national TOrC mitigation strategy. Even the Swiss national strategy with a strong focus on reduction of compound emissions from point sources may not be applicable in areas with intensive agricultural use (and associated non-point source discharges) or where wastewater contributes only a small portion to the pollution of a particular water body. While the Swiss approach of upgrading mainly wastewater treatment plants in the densely populated northern part of the country are expected to result in a noticeable load reduction of TOrC, adopting the same strategy nationwide in countries covering large and diverse geographic areas as the U.S., Australia, the EU or Germany may not be a viable option. The control of TOrC emissions from non-point sources is extremely challenging and although various mitigation strategies are available, their efficiencies have to be evaluated (Reichenberger et al., 2007). An alternative approach for such countries may be to identify “hot spots” of elevated TOrC concentrations and associated adverse health effects in receiving streams, which could then be prioritized in point source reduction programs.

Considering the variety of management strategies in the targeted countries, it may be preferable to define specific water quality standards for watersheds rather than aiming for a pre-defined relative minimum degree of TOrC removal at a specific point source (as favored in Switzerland). Risk assessment approaches, such as those applied in Australia, allow tailoring approaches and mitigation strategies to local conditions while requiring a comprehensive analysis of site-specific factors. In particular for federally structured countries or hierarchically organized unions, a suitable approach may be to integrate nationwide standardized strategies with measures tailored to local conditions as practiced in the EU. Here, the combination of mandatory and supplementary measures ensures a common EU-wide basis for the management of TOrC while leaving the individual member countries with the flexibility to design strategies tailored to regional watershed conditions.

Beyond the regulatory development and enforcement of water quality standards, the USA has numerous programs, designed to generate new toxicity and/or occurrence data and to conduct research and development for new water treatment technologies, analytical method development, and novel approaches to health assessment. Of these, the most applicable to water is the USEPA’s Office of Research & Development (ORD). The ORD conducts numerous research projects related to water testing, treatment, and toxicity assessment and provides financial grants to other organizations through a variety of competitive programs. As examples, the ORD led the ToxCast program, which investigates numerous aspects to high-throughput biological assay screening and adverse outcome pathways (Judson et al., 2010; Kavlock et al., 2012). These are very important to water as many high-throughput *in vitro* bioassays are readily applicable to water quality monitoring, though the initial research is more focused on discrete chemical testing. The ORD also leads the Endocrine Disruption Screening Program (EDSP), which is investigating the endocrine disruptive potential of chemicals and mixtures that may contaminate U.S. food and water (Snyder et al., 2003; USEPA, 2011b). In addition, the USEPA interacts with the U.S. Geological Survey (USGS) who conducts numerous monitoring programs for water and environmental matrixes for emerging contaminants as well as conducts aquatic health studies. The USGS proposed two effects- and exposure-driven risk assessment approaches for transformation products of water contaminants, which help to evaluate the risk potential of single transformation products or mixtures (Murphy et al., 2012). There are many other programs that include R&D efforts of U.S. federal agencies highly relevant to the monitoring, treatment, and health characterization of emerging contaminants, but these are beyond the scope of this manuscript and may be found in other references cited (Kolpin et al., 2002; Burkholder et al., 2007; Focazio et al., 2008; Knight et al., 2009; Martin et al., 2010; Tice et al., 2013; Kleinstreuer et al., 2014; Snyder, 2014).

4.5 Conclusions

The wide range of potential pathways of compounds into the environment, heterogeneous and site-specific occurrence pattern of TOrC, regional diversity in compound usage, population density, differences in water and land usage, and other influencing factors have to be considered when developing and specifying management strategies for TOrC. While it has been recognized that regional-specific conditions and various factors should be considered, managing TOrC comprehensively in water remains a challenge. Approaches adopted for managing TOrC in the aqueous environment differ among countries due to different underlying regulatory philosophies, socio-economic factors, environmental conditions, and environmental policy legacies. Also, countries follow different identification and prioritization procedures for selecting priority TOrC for monitoring efforts and regulatory consideration, ranging from conducting comprehensive screening efforts as part of the 'Contaminant Candidate List' in the USA for drinking water contaminants to a short list of 12 defined TOrC performance indicators to assess the efficiency of mitigation measures in Switzerland. Screening efforts to identify either human or environmental health relevant chemicals among the different countries also follow different procedures. Strategies are generally either based on toxicity threshold values or emission avoidance or a combination of the two approaches. Toxicity-based strategies are compound-specific and provide a quantifiable amount of safety from adverse effects caused by known compounds. Toxicity-based strategies are not suitable to properly addressing the risk associated with transformation products or not yet identified TOrCs in water sources, because of insufficient toxicological information. While these approaches tolerate the presence of these compounds in the environment below their threshold levels, they also only capture a limited number of identified chemicals with known occurrence and toxicity data. Strategies following the emission avoidance principle are aiming to prevent the release of TOrCs into the aqueous environment in general. This approach assumes that by minimizing compound concentrations in receiving water bodies potential adverse effects of compounds on the aquatic environment or on human health are prevented. For this strategy, a toxicological assessment of single compounds is not required and measures might also mitigate the release of TOrCs which have not been identified yet (e.g., transformation products). Broadly defined relative removal goals for TOrC discharge from point sources, as applied in emission avoidance-based strategies like in Switzerland, are coupled to meeting concentration levels in receiving streams that are below save exposure levels. Due to varying TOrC influent concentrations of wastewater treatment facilities and different flow conditions in the receiving stream, meeting pre-defined removal percentages cannot guarantee that health-relevant threshold concentrations will not be exceeded. Also, the concept of defining general blanket values (e.g. 0.1 µg/L) is not ubiquitously applicable, because thresholds of compounds can differ and some compounds may show adverse effects on environmental health even in the sub-nanogram concentration range.

For a comprehensive TOrC management it is necessary to advance current strategies and develop new frameworks, considering additional parameters, such as social or economic interests. An advanced framework has recently been published, expanding common management concepts and including mitigation strategies and social aspects in management strategies (Munthe et al., 2017). Due to the wide range of TOrCs and the various pathways into the environment, only a comprehensive combination of different strategies may result in a balanced, flexible, and socially and economically acceptable policy program. A combined approach of toxicity-based and emission avoidance tailored to local conditions can allow managing the risks posed by TOrC on two levels: Risks from known hazardous compounds are reduced by enforcing toxicologically-based concentration values and unknown compounds and compounds with unknown health effects are minimized by reducing their release into the aquatic environment. As a result, both environmental and human health are protected.

The tested hypothesis that all management strategies for TOrCs consist of toxicity- and/or emission avoidance-based principles can be accepted.

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Chapter 5 - An assessment of international management strategies for TOrCs in water[2]

This study investigated management strategies among different countries worldwide (USA, Australia, Switzerland, EU, and Germany) for mitigating the risk associated with trace organic chemicals (TOrCs) in the aqueous environment. Although national strategies are adapted to specific geographic conditions and consider local occurrence pattern of TOrCs, two basic principles for reducing the release of chemicals could be identified among different countries. Toxicity-based strategies rely on regulating maximum allowable concentrations, which intend to limit the release of specifically known hazardous compounds to the aqueous environment. Strategies based on the emission avoidance aim to minimize the occurrence of undesired trace organic compounds in water bodies in general regardless of an identified risk. Both principles were implemented by suitable measures, which allowed minimizing or reducing the concentration of compounds in water bodies. Such measures can target single chemicals or groups of chemicals. Although strategies and implemented measures for the management of TOrCs were multifold, all strategies relied on comprehensive monitoring programs using target analysis for TOrCs. However, non-target and suspects screening analyses are playing an increasing role in identifying relevant chemicals for inclusion in future monitoring programs.

This publication tested the hypothesis that toxicity and emission avoidance based strategies use the same set of analytical tools to assess the efficiency of implemented measures and to monitor known and identify unknown compounds.

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[2] Bieber, S., Rauch-Williams, T., Drewes, J.E., 2016. An Assessment of International Management Strategies for CECs in Water, in: Drewes, J.E., Letzel, T. (Eds.), Assessing Transformation Products of Chemicals by Non-Target and Suspect Screening − Strategies and Workflows Volume 1. American Chemical Society, Washington, DC, pp. 11–22. doi:10.1021/bk-2016-1241.ch002

Chapter 5 - An assessment of international management strategies for TOrCs in water

5.1 Introduction

Nearly all human activities result in the release of chemicals into the environment. The spectrum of these compounds is very broad, reaching from carbon dioxide formed in cellular respiration to synthetic chemicals, produced in industrial processes. With currently more than 110 million registered chemical compounds and several thousand in daily use, it is no surprise that many of these compounds enter the environment by accident or after their intended use (CAS.org, 2016). The occurrence of organic chemicals in the aqueous environment is of high concern, since many of these constituents can potentially adversely affect human and environmental health including pesticides, biocides, pharmaceuticals, hormones, or household chemicals (Richardson and Ternes, 2014). Although such compounds are commonly detected in the nanogram per liter (ng/L) to microgram per liter (µg/L) concentration ranges, the compound specific mode of action may remain active and pose a potential risk to environmental or human health. Adverse effects on aquatic health by chronic exposure by trace organic compounds have been documented previously and also mixtures of several compounds can result in adverse health effects (Faust et al., 2001; Schwaiger et al., 2004; Triebskorn et al., 2004; Fent et al., 2006). Through use of impaired water sources for drinking water supply, trace organic compounds might also pose a risk to human health (Stackelberg et al., 2004). In addition to compounds, being discharged to the aquatic environment, transformation processes formed in the environment and engineered water treatment processes, can contribute new compounds with unknown characteristics (Agertved et al., 1992).

General concerns and proven adverse effects of trace organic compounds (TOrCs) regarding environmental health have resulted in management strategies and legislative action in many countries worldwide. Although, these countries share the same concern, the proposed national management strategies are rather heterogeneous due to differences in environmental, geographic and economic conditions and different opinions regarding relevant health endpoints targeted to protect species from adverse effects by trace organic compounds. Nevertheless, the core principles of these different approaches can be elucidated providing an opportunity to identify promising and effective strategies in managing the risk from trace organic chemicals in the aqueous environment.

5.2 Principles of management strategies

The occurrence of TOrCs associated with potentially adverse health effects in waterbodies are an international challenge (Malaj et al., 2014). The occurrence patterns of these compounds, however, can be highly variable across different countries and depend on population density, land-use, wastewater dilution in streams, compound usage and others factors. These factors provide the framework in which national and regional management strategies have to be adopted. Most commonly, management strategies aim to protect environmental health and/or human health against adverse effects from trace organic compounds. These strategies are directed to manage TOrCs in the entire water cycle and can either be immission- or emission-driven. Immission-driven strategies intend to preserve or restore a desired environmental state of a receiving water body whereas emission-driven strategies intend to minimize discharges from point and non-point sources. The specific targets for both strategies can be based on specific evidence for adverse effects that allows deriving a safe risk level or are motivated by considerations following the precautionary principle to achieve a general reduction of specific chemicals. The following sections investigate what drivers have resulted in the development of different management strategies for TOrCs in different countries worldwide including the United States of America (U.S.), Australia, Switzerland, the European Union (EU), and Germany.

Australia has suffered from extreme weather phenomena in the last two decades resulting in extended periods of severe water scarcity and extreme flooding. As a result, water reuse schemes have been considered and implemented to reduce pressure on drinking water supply during times of limited supplies. In order to manage risks associated with potable water reuse practices, a strategy following the World Health Organizations guidelines for drinking water was implemented in Australia (WHO, 2004; NWQMS Phase 1, 2006; NWQMS Phase 2, 2008). This strategy is based on a hazard analysis and critical control point concept (HACCP). All processes involved in water reuse and drinking water supply have to be evaluated and potential risks and critical points of the entire supply system (from source, over treatment to final use) identified. Such points or process steps are defined as critical control points and specific procedures for risk minimization are implemented. The prevention of hazardous events and risk exposures associated with them are ensured by the implementation of multiple barriers (NWQMS Phase 2, 2008). Organic and inorganic chemicals are regarded as potential hazards for human health and the Australian guidelines for water recycling provide health-based guideline values for certain trace organic compound (NWQMS Phase 2, 2008). These values are non-enforceable and should only provide orientation values regarding safe levels for compound concentrations. State governments, which have the competence to implement enforceable quality standards, can choose different compounds and compound concentrations as threshold than those provided in the guidelines.

Health-based guideline values as well as enforceable MAC values are intended to provide a measurable amount of safety of exposure for selected compounds. Such values can be derived by toxicity testing of sensitive species or predictive models like quantitative structure–activity relationships (QSAR) (Escher et al., 2006; European Commission, 2012). Safety factors can be applied on effect concentrations in order to provide an appropriate and measurable degree of safety (European

Commission, 2011) Guideline and MAC values can be used for the protection of both, human and/or environmental health. Values targeting the protection of human health can be applied for surface, ground- or drinking water, while those for environmental health are mainly applied in surface waters or related waterbodies.

The concept of enforceable MAC values among others is applied in the U.S. and the EU. Trace organic compounds are assessed regarding their toxicological relevance and threshold level concentrations are determined. The EU Water Framework Directive (WFD) provides MAC values for the protection of environmental health in surface ground and coastal waters (European Commission, 2000). In the U.S., emerging contaminants in drinking water are identified and prioritized within the framework of the Unregulated Contaminant Monitoring Rule (UCMR) and the Contaminant Candidate List (CCL) (USEPA, 2009, 2012b).

The EU has implemented a comprehensive regulative framework, aiming to prevent the emission of trace organic compounds to the aquatic environment covering the entire life cycle of chemicals from authorization, over production and distribution to disposal (European Commission, 2000, 2006a, 2010). The instrument of MAC values is applied for chemicals identified as hazardous compounds. The classification as a hazardous compound results in a mandatory reduction of compound emissions, but might also trigger complete phase out (European Commission, 2000). The EU WFD requires individual member states to define river basin districts, to evaluate the chemical and ecological status of water bodies, and to specify a program of measures (PoMs) to reach a certain condition within these river districts. The main goal of the WFD is to restore a natural state in all waterbodies, showing no or only “low levels of distortion resulting from human activity” by specific target dates (European Commission, 2000). The requirements for good chemical and good biological status are defined and environmental quality standards (EQS) are set for specific contaminants in either aqueous or tissue samples.

The currently most ambitious and thoroughly planned strategy for the management of trace organic compounds is implemented in Switzerland. Trace organic compounds are already part of the national water quality regulation (Schwarzenbach et al., 2006). The Swiss strategy aims to minimize the emission of trace organic compounds into the aqueous environment in general (Federal Assembly of the Swiss Confederation, 2016). All compounds of anthropogenic origin, which can be detected in environmental waterbodies, are regarded as potential threat to environmental health and concentrations should be minimized. Toxicologically derived MAC values are not commonly applied in Switzerland, but threshold values can be assigned for different compound groups. Such values are applied for pesticides in groundwater, where single compounds shall not exceed 0.1 µg/L and the sum of all pesticides should be below 0.5 µg/L (Swiss Federal Council, 2016). The Swiss national strategy includes upgrading 100 of the approximately total number of 700 wastewater treatment plants with advanced water treatment processes (i.e., either ozonation or activated carbon filtration) over a period of 40 years, targeting to treat approximately 50% of the entire wastewater flow generated in Switzerland after full implementation of the program (Eggen et al., 2014). The efficiency of measures for compound emission reduction in wastewater treatment plants is assessed by monitoring a subset of twelve indicator compounds. Criteria for the selection of these performance indicators were ubiquitous occurrence in Swiss surface and wastewater, insufficient biodegradability and removal efficiency by ozonation or activated carbon (Götz et al., 2015). Effects of emission reduction measures on receiving streams will be monitored, too.

At first glance, quite diverse strategies to manage trace organic compounds in the aquatic environment are the two main underlying principles. Toxicity-based strategies rely on MAC values for hazardous compounds derived from their toxicological relevance, which provide safety against adverse effects for human or environmental health, if concentrations in environmental compartments do not exceed proposed values for specific chemicals. Strategies based on the precautionary principle aim to prevent the emission of compounds into the environment regardless of a proven health-relevance level. Toxicity-based strategies are well suitable for the control of known, hazardous trace organic compounds and are, among others, implemented in the United States, Australia, and the EU (European Commission, 2000; NWQMS Phase 1, 2006; NWQMS Phase 2, 2008; USEPA, 2014a, 2014b). Procedures for the derivation of such quality standards and determined concentrations are usually summarized in specific national regulations.

To verify conformity with legal requirement, measured environmental concentrations (MECs) are compared to maximal allowable concentrations. In case of exceedance of allowed concentrations in water bodies, measures for the reduction of environmental compound concentration have to be implemented. Although the toxicity-based approach is readily applicable for previously detected and identified trace organic compounds, it cannot be used to control previously not detected and unknown compounds. A toxicological assessment of all compounds, detectable or possibly contained in environmental water samples is not practicable, due to high costs and time consumption. Possible adverse effects caused by mixture toxicity of several compounds, including transformation products which co-occur in waterbodies have to be assessed separately (Escher and Fenner, 2011).

Strategies based on the precautionary principle do not rely solely on compound specific toxicity assessments to preserve a desired water quality. Based on the concept that chemical compounds of anthropogenic origin do not belong into the environment and all compounds possibly may pose a risk to the environment, the emission of compounds into the environment in general should be prevented.

A complete elimination of compound emissions is in most cases not realistic, thus strategies that can result in a significant emission reduction are favored. While for some strategies a toxicological assessment of individual chemical compounds is not intended, groups of compounds or compounds with known mode of action can be assigned threshold values. These threshold values are typically generic values and not based on toxicological data. Such criteria have been defined to minimize discharge of pesticides to drinking water sources in the European Union and in Switzerland (European Commission, 2006b; Swiss Federal Council, 2016). If stricter values for a certain compound are defined in other regulations, these have to be applied. Compared to toxicity-based strategies, emission avoidance-based strategies are more difficult to implement. This is because not all pathways compounds can take to enter the environment are known or can be effectively managed. While point source emissions from wastewater treatment plants can be reduced through implementing advanced wastewater treatment technologies, non-point sources are more difficult to locate and restrict. A reduction of compound emissions does not only result in a reduced risk posed by known compounds, but also by unknown or undetected compounds. Additionally, the risk from transformation products being formed can be reduced, too. Switzerland has implemented a management strategy, which is entirely grounded on the emission avoidance (Federal Assembly of the Swiss Confederation, 2016). The strategy to manage CECs in the European Union combines elements from both described principles (European Commission, 2000). Maximal allowable concentrations, derived from toxicity assessments are determined for known hazardous compounds (toxicity-based). For these compounds emissions have to be reduced or the chemicals are phased out entirely. As a second part of the strategy, a reduction of compound emissions in general is aimed by defining water quality status of river districts that restore a natural state to benefit aquatic life but also humans (precautionary principle).

5.2.1 Mitigation measures

Implementing strategies to reduce TOrCs discharge to the aquatic environment have to be accompanied by measures and tools allowing to control or reduce trace organic compound emissions or environmental concentrations effectively. The impact of certain measures can reach from the reduction of single compound emissions to a removal of a broad spectrum of compounds. The character of an implemented measure depends upon the realized core principle and the health endpoint, which should be protected against adverse effects, caused by certain compounds. Measures for the protection of drinking water can target compound emissions into source waters or be implemented in drinking water utilities. For the protection of the aquatic environment, measures have to target the emission of compounds.

5.2.2 Compound specific measures

Source control measures might be motivated by both toxicity-based strategies and emission avoidance for single compounds. The regulation of compounds or restrictions of compound usage are an option to reduce or phase out emissions of hazardous compounds into the environment. Developing regulations for chemicals can be impeded by stakeholder opposition including manufacturers or users of compounds. For compounds like pharmaceuticals, regulation restricting their use is problematic due to ethical reasons. The substitution of hazardous compounds by compounds with comparable mode of action but lower hazardous potential is another option to reduce or phase out emissions of a specific compound. Suitable substitutes might not always be available and potential alternative compounds have to be toxicologically assessed before substitution to ensure a true reduction of overall health risks. Measures involving participation of consumers, like voluntary waiver of compound usage or incentive systems, require a high degree of public information and outreach. While regulation can result in an immediate and complete phase out of compound emissions into the environment, the impact of voluntary emission reduction measures cannot be predicted and depends on several factors.

5.2.3 Technological measures

Technological measures can be applied for emission reduction of a broad compound spectrum. Such measures are often favored over source control programs due to easier implementation, but are limited in reducing the emission of compounds from non-point sources. Advanced wastewater treatment as compound emission reduction measure is therefore proposed and implemented in select river districts across Europe and Switzerland (European Commission, 2000; Eggen et al., 2014). In these countries, the implementation of advanced wastewater treatment prior to discharge in receiving streams is a consequence of following the emission avoidance principle or meeting specific EQS values for the protection of the environment. Powdered or granular activated carbon and ozonation are currently evaluated and implemented for the emission reduction of trace organic compounds due to their applicability, removal/reduction efficiency and robustness (Hollender et al., 2009; Abegglen and Siegrist, 2012; Eggen et al., 2014). Advanced oxidation processes (e.g., UV/H_2O_2 or Fenton/H_2O_2) might be suitable for the removal of trace organic compounds, but applicability of these techniques at full-scale still has to be demonstrated (Ureña de Vivanco et al., 2013). Technologies used in wastewater treatment plants for the emission reduction can also be applied in drinking water utilities, but serve there mainly to protect human health.

The efficiency of technological measures for the reduction of compound emissions can be assessed by monitoring of performance-based indicator compounds through the reduction/removal process (Dickenson et al., 2009; Götz et al., 2015). Ideally, indicator compounds are present in untreated samples and can be detected and monitored through the whole treatment process. Adsorptive removal techniques like powdered or granular activated carbon are most suitable for the removal of medium to non-polar compounds. Some polar compounds can partly be removed by activated carbon techniques (Kovalova et al., 2013). Ozonation and advanced oxidation processes (AOP) can result in the formation of transformation products which tend to be more polar than the parent compounds (Boxall et al., 2004). Transformation products, resulting from oxidation or biological treatment techniques, display an uncertainty since identity and toxicological potential are often unknown. The possible formation of oxidation by-products, which might be toxicologically relevant, also has to be considered before implementation (Hollender et al., 2009). As a consequence, the spectrum of detectable compounds can change after implementation of a specific removal process.

5.3 Water quality monitoring as a cornerstone of all management strategies

While the core principles of national strategies can be diverse, comprehensive monitoring of TOrCs in environmental water bodies and drinking water is implemented in all countries investigated in this study. For strategies following both toxicity-based and emission avoidance-based approaches, target analysis, suspects screening and non-target screening methods are employed, although to a different extent (Figure 5-1). Monitoring TOrCs can serve the function of triggering the implementation of measures to control compound emissions in the future and for compliance to monitor the success of already implemented measures. The analysis of TOrCs in water samples is commonly conducted using gas (GC) or liquid chromatography (LC), coupled to mass spectrometric detection (Richardson and Kimura, 2016). Detection and quantification of known compounds is achieved by target screening using (isotope labelled) reference substances (Letzel et al., 2015). For the identification and monitoring of unknown compounds non-target and suspects screening are employed (Letzel et al., 2015). Non-target screening aims to identify detected masses derived from instrumental analytics. This approach can be ambiguous, since one mass can account for numerous different compounds with (nearly) identical masses, but different formulas or chemical structures. Thus, while no quantitative data can be derived, non-target screening approaches can serve as a comparative fingerprint analysis or assist in the identification of unknown trace organic chemicals. Suspects screening utilizes additional information for the analyzed sample in order to identify compounds. However, a final verification of compound identity can solely be made using target screening involving reference substances.

In toxicity-based strategies, target-screening is used to compare measured environmental compound concentrations with MAC values. For emission-avoidance based strategies, compliance of environmental compound concentrations with generic limiting values is proven by target-screening, too. In order to detect the occurrence of new potentially hazardous emerging contaminants in water bodies, monitoring cannot solely be based on target-screening. Non-target and suspects screening approaches are utilized when certain masses are frequently detected in water samples. Information like the origin of a sample or applied water treatment processes, but also specific databases can assist in classifying chemicals into suspect compound groups or classes. Where advanced (oxidative) treatment technologies were applied for treatment of wastewater or surface water, water samples possibly contain transformation products. This information can be used to generate lists of common oxidative transformation products, which are likely to be present in a sample. Based on such lists, mass spectrometric data can be searched for matching masses, which might indicate the presence of a compound. For the final verification of a compound's identity, reference substances have to be obtained. Such monitoring strategies are already part of some national regulations and provide the basis for the monitoring of emerging contaminants, as the Watch List under the European Water Framework Directive or the Unregulated Contaminant Monitoring Rule (UCMR) and Contaminant Candidate Lists (CCL) under the United States Safe Drinking Water Act (USEPA, 2009; Snyder, 2014; European Commission, 2015a; Letzel et al., 2015; Richardson and Kimura, 2016).

Suspects and non-target screening play an important role in monitoring strategies already, but will likely become even more important in the future. The raising awareness about the presence of trace organic compounds and the use of advanced water treatment processes for their mitigation in wastewater, water reuse, and drinking water applications require more comprehensive monitoring strategies to assess the safety and quality of different water types. The changing polarity spectrum of detectable trace organic compounds including transformation products of chemical and biological treatment processes towards higher polarity, more appropriate requirements for instrumentation conditions and alternative chromatographic techniques have to be evaluated. Additional information sources, which can provide support for the identification process of detected compounds, will gain increased interest. With several thousand detectable masses in a water sample, all available information should be utilized to reduce the number of compounds present in a sample to those of environmental relevance. Although occurrence patterns of trace organic compounds can differ by region, these contemporary monitoring strategies can be applied irrespective of location. Therefore, screening strategies and identification processes should be harmonized on a global basis. This will also improve comparability of results between different institutions and save resources in developing appropriate analytical approaches at an individual national level.

The hypothesis that toxicity and emission avoidance based strategies use the same set of analytical tools to assess the efficiency of implemented measures and to monitor known and identify unknown compounds can be accepted.

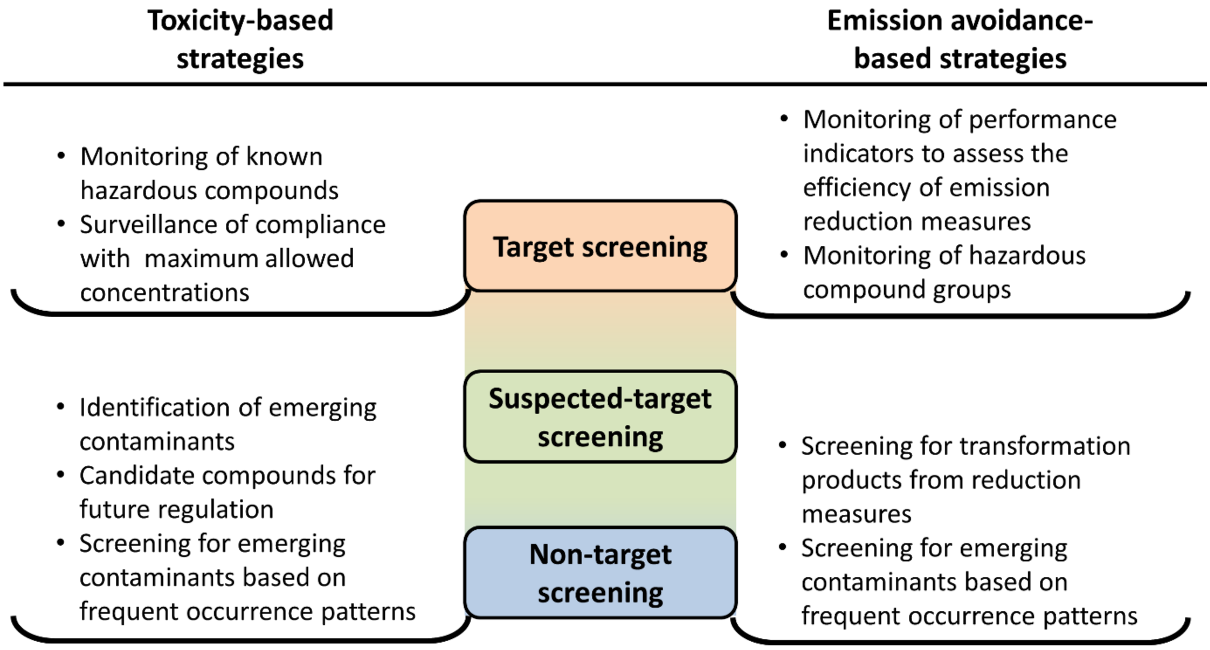

Figure 5-1: Different screening strategies for trace organic chemicals in water samples following different management principles.

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Chapter 6 - RPLC-HILIC and SFC with mass spectrometry: polarity-extended organic molecule screening in environmental (water) samples[3]

Trace organic compounds are important in environmental analysis, because they impact water quality and introduce potential (eco)toxicological effects. Current analytical methods mostly rely on gas chromatography (GC) or reversed-phase liquid chromatography (RPLC) coupled with (tandem) mass spectrometry. However, neither method can easily separate very polar molecules. This study presents, two chromatographic separation strategies, a serial RPLC- hydrophilic interaction liquid chromatography (RPLC-HILIC) coupling and an analytical scale supercritical fluid chromatography (SFC) system, and validates their separation effectiveness as polarity-extended chromatographic methods for 274 environmentally relevant compounds. Compounds tested were grouped into three polarity classes, "very polar" {log D (pH 7) below -2.5}, "polar" {log D (pH 7) -2.5 to +2}, and "non-polar" {log D (pH 7) higher than +2}). Nearly all compounds could be retained in both systems with relative standard deviations of retention times (RT) (n = 6) typically between 2 and 5%. Both techniques have considerable benefits when combined with accurate mass spectrometric detection. Molecules RT and accurate mass were recorded in a database for each set up. This information was used for compound screening measurements like "hidden-target screening" in complex environmental matrices (such as wastewater treatment plant effluents). Results of both techniques are complementary and useful for all types of molecules polarity. In this study, more than 80 percent of the compounds found in wastewater treatment plant effluent samples possessed a negative log D (pH 7) value. This result highlights the basic necessity to include "very polar" compounds in water monitoring techniques and protocols.

This publication tested the hypothesis that polarity extended separation techniques widen the view on TOrCs present in the aquatic environment, by detecting more polar environmentally relevant compounds in water samples than reversed phase LC-MS.

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[3] Bieber, S., Greco, G., Grosse, S., Letzel, T., 2017. RPLC-HILIC and SFC with mass spectrometry: polarity-extended organic molecule screening in environmental (water) samples. Anal. Chem. 89 (15), 7907–7914. doi:10.1021/acs.analchem.7b00859

Chapter 6 - RPLC-HILIC and SFC with mass spectrometry: polarity-extended organic molecule screening in environmental (water) samples

6.1 Introduction

The presence of anthropogenic organic compounds in the environment including surface waters, ground water, air, soils, and within biota is frequently reported. For instance, molecule concentrations have been detected in the ng/L to µg/L range in surface waters (Heberer, 2002; Kolpin et al., 2002; Nakata, 2005; Hughes et al., 2012; Richardson and Ternes, 2014; Sengupta et al., 2014). Trace organic compounds increase in importance, because they originate from pharmaceuticals, personal care products, pesticides, and herbicides and enter the environment mainly via wastewater treatment plants or surface run-offs (Ternes, 1998; Benotti et al., 2009). Acute toxic effects of trace organic compounds on human health are not expected at these low concentrations (Enick and Moore, 2007). However, long-term exposure of aquatic organisms to sublethal concentrations, similar to those found in surface waters, can cause chronic health effects (Fent et al., 2006; Wernersson et al., 2015) and may influence the ecosystem (Brack et al., 2016). Many countries have reacted to this concern regarding the potentially adverse effects to the environment by amending their water regulations. A central part of most management strategies is comprehensive monitoring of the trace organic compound occurrence in waterbodies (Bieber et al., 2016a). In the United States, the clean water act (CWA) and safe drinking water act (SDWA) regulate water quality. Trace organic compounds are included in this regulatory framework mainly through unregulated contaminant monitoring rules (UCMR) (USEPA, 2012b) and the drinking water contaminant candidate list (CCL) (USEPA, 2009). The European Commission amended the water framework directive (European Commission, 2000) (2000/60/EC) in 2013 to include a watch-list for pollutants that “may pose a risk to, or via the aquatic environment and for which monitoring data is not sufficient” (European Commission, 2013) to the list of monitored pollutants. The first version of this watch-list includes estrone (E1), 17-beta-estradiol (E2), 17-alpha-ethinylestradiol (EE2), macrolide antibiotics, and diclofenac (European Commission, 2015a). This is the first attempt within the European Union to include pharmaceuticals and hormones in routine water monitoring. However, waterbodies likely carry a large proportion of polar and very polar organic compounds due to the very polar nature of water. Unknown trace organic compounds and their transformation products (TPs) can appear in the environment, in addition to known polar molecules. TPs are often more polar than the original compound (Boxall et al., 2004). Current water quality or pollutant monitoring is mostly performed by liquid chromatography (LC) – (tandem) – mass spectrometry (MS) using reversed phase (RP) columns for chromatographic separations (Vanderford and Pearson, 2003; Vieno et al., 2005; Schymanski and Singer, 2014). Although RPLC-MS is an established method for the routine monitoring of these regulated and characterized trace organic compounds, it is limited to non-polar and moderately polar molecules. For the identification and monitoring of very polar compounds alternative separation techniques are required (Reemtsma et al., 2016).

The development of a separation technique with an extended polarity range would allow simultaneous monitoring of non-polar, polar, and very polar compounds. The nomenclature of “polarity” is defined in the presented study via log D values (Kah and Brown, 2008) similar to the following: ”very polar” is at log D <-2.5, “polar” at log D -2.5 to log D +2 and “non-polar” at log D >+2. Currently, non-polar and polar compounds (log D (pH 7) > -2.5) can be easily retarded and separated using “non endcapped”, “polar endcapped” or “polar embedded” RPLC columns. Thus, both polarity classes (non-polar and polar) can be monitored in one run within one column. The separation of very polar (log D (pH 7) < -2.5) compounds is restricted to normal phase liquid chromatography (NPLC), ion chromatography (IC), capillary electrophoresis (CE), and hydrophilic interaction liquid chromatography (HILIC).

An ideal chromatographic system should provide the separation of non-polar, polar, and very polar compounds in a single run. Such systems are represented by 2-D separation techniques using NPLC/RPLC(Ahn et al., 2011), IC/RPLC (Brudin et al., 2010), HILIC/RPLC (Xing et al., 2012), and the parallel coupling (Klavins et al., 2014) or serial coupling of an RPLC column and a HILIC column (Greco et al., 2013). RP columns can be used to separate “polar” and “non-polar” compounds. HILIC effectively separates polar and very polar molecules and has already been used to separate polar environmentally relevant compounds (Greco et al., 2014; Tang et al., 2016). The orthogonality of these chromatographic techniques enhanced by the same type of mobile phases and has been applied in various disciplines (Chalcraft and McCarry, 2013; Greco et al., 2013, 2014; Rajab et al., 2013; Chen et al., 2016).

Supercritical fluid chromatography (SFC) was introduced in the early 1960s, but took 20 years to gain acceptance in analytical separation sciences (Klesper et al., 1962; Saito, 2013). The strength and reliability of SFC has increased substantially over the last five years because several vendors have introduced robust analytical SFC systems. The mobile phase of SFC is typically comprised of compressed carbon dioxide (CO_2). Liquid solvents such as methanol are commonly used to optimize SFC separation and to moderate the elution of compounds from the stationary phase. SFC is considered as a green technology because of its relatively low consumption of organic solvents (Taylor, 2009). In addition to the use of SFC for the separation of chiral and achiral molecules in pharmaceutical industry, SFC can also separate small molecules very effectively from complex mixtures (Berger and Wilson, 1993; Francotte, 2001; Weller et al., 2010; West, 2014; Bieber and Letzel, 2015e).

The serial RPLC-HILIC coupling and SFC, coupled via electrospray ionization with an accurate high resolution mass spectrometer (like time-of-flight; i.e. ESI-TOF-MS) provide the especially important benefit of extending the range of separable and detectable compounds from “non-polar” to “polar” and even "very polar". This study validated this broader view on trace organic compounds by comparing RTs and accurate masses of a set of 274 standard compounds. Retention data were used to investigate the separation capabilities of both techniques and to create an analytical database. Subsequently, the database was used for the data analysis of wastewater treatment plant effluent samples using the "hidden target screening" strategy, which does not require tandem mass spectrometry and proving the applicability of polarity extended separations (Letzel et al., 2015; Letzel and Drewes, 2016). A comparison of both techniques leads to novel insights regarding polarity-extended chromatographic separations in environmental research.

6.2 Experimental section

Acetonitrile and methanol, both HiPerSolv Chromasolv LC-MS grade, were purchased from VWR (Darmstadt, Germany) and LC-MS Chromasolv water was bought from Fluka (Buchs, Switzerland). Carbon dioxide (CO_2, purity 4.5) was obtained from Westfalen AG (Muenster, Germany). Ammonium acetate was purchased from Sigma-Aldrich, (Seelze, Germany). Standard compounds were obtained from ACROS organics (Thermo Fisher Scientific, Geel, Belgium), Alfa Aesar (Thermo Fisher Scientific, Karlsruhe, Germany), Cayman Chemical Company (Ann Arbor, Michigan, USA), CHEMOS GmbH (Regenstauf, Germany), Dr. Ehrenstorfer (Augsburg, Germany), Merck KGaA (Darmstadt, Germany), Fluka (Buchs, Switzerland), Sigma-Aldrich (Seelze, Germany), Supleco (Bellefonte, Pennsylvania, USA), and TCI (Eschborn, Germany). The polarity of standard compounds ranged from "very polar" (i.e., logD (pH 7) -7.71) to "non-polar" (i.e., logD (pH 7) +7.67). Further details are summarized in Table S- 7 on page 179.

6.2.1 Standard compounds and working mixtures

Polar and very polar standard compounds were dissolved in acetonitrile/water (50/50, v/v), and non-polar compounds were dissolved in acetonitrile that resulted in 1 mM stock solutions. Very polar, polar, and non-polar compounds were combined into working standard mixtures with a final concentration of 10 µM per compound in acetonitrile/water (50/50, v/v).

6.2.2 RPLC-HILIC setup

The coupling set-up (Figure 6-1a) and system parameters were based on a previously reported method (Greco et al., 2013). The current study modified some of these. Specially, two Agilent 1260 Infinity LC-systems that each contained a binary pump, an on-line degasser and a mixing chamber were used. The reversed phase separation was performed with a non-polar endcapped Poroshell 120 EC-C18 (50.0 × 3.0 mm, 2.7 µm; Agilent Technologies) column. The HILIC separation was conducted with a ZIC®-HILIC column (150 × 2.1 mm, 5 µm, 200 Å; Merck Sequant, Umea, Sweden). The columns were coupled through a T-piece (Upchurch, IDEX Europe GmbH, Erlangen, Germany). The third port of the T-piece was connected to a second binary pump (Figure 6-1a). The mobile phase for the RPLC consisted of 10 mM ammonium acetate in water/acetonitrile (90/10, v/v) and 10 mM ammonium acetate in water/acetonitrile (10/90, v/v, pH value is about pH 7). The solvents acetonitrile and water were used for the HILIC separation. Data about mobile phase gradients are provided in Table S- 8 (p. 193). The injection volume was 10 µL.

6.2.3 SFC

An analytical Agilent SFC system (Agilent Technologies, Waldbronn, Germany) consisting of a binary pump, an auto sampler, a column oven and diode array detector (DAD), equipped with a zwitterionic HILIC column (150 x 2.0 mm, 5 µm, KNAUER, Berlin, Germany) was used for the SFC analyses (Figure 6-1b). A binary gradient of CO_2 and 20 mM ammonium acetate in methanol (modifier) was used to promote compound elution. Column temperature was held constant at 40 °C and system backpressure was set to 150 bar in all experiments (see BPR in Figure 6-1b). Starting condition of the binary gradient were 95% solvent A (CO_2) and 5% B (modifier). After a short isocratic stage, modifier content was increased to 40% B. Flow rate was held constant at 1.5 mL/min. Details about the chromatographic conditions are summarized in Table S- 9 (p. 193). The injection volume was 10 µL. The void time of the SFC with MS detection was determined to be less than 0.6 minutes. For the calculation of retention factors (k) a void time of 0.6 minutes was used.

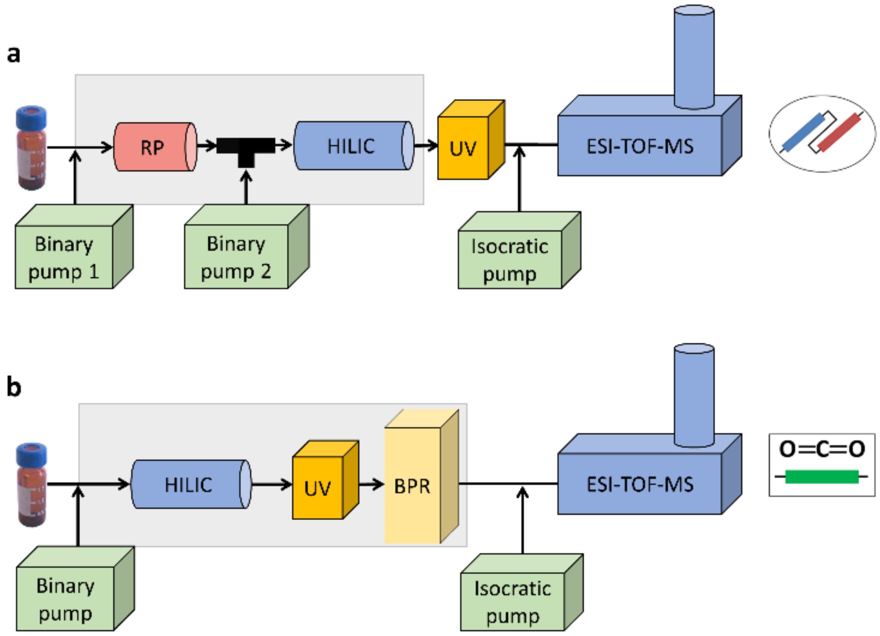

Figure 6-1: RPLC-HILIC coupling scheme (a) and the SFC system (b). Both systems were coupled to a time-of-flight mass spectrometer. Both configurations used an isocratic pump to deliver an electrospray-ionization enhancing solvent flow and mass correction standards. Grey boxes represent the pressurized region. The SFC system includes the UV detector in the high-pressure area that is mediated by the backpressure regulator (BPR). Identifiers are used in the following accordingly.

6.2.4 Mass spectrometry

The flow of the chromatographic systems and an isocratic pump (Agilent Technologies, Waldbronn, Germany) were mixed via a T-piece and connected to the inlet of the Jet Stream ESI interface of an Agilent 6230 TOF-MS (both Agilent Technologies, Santa Clara, CA, USA). The isocratic pump added reference solution that consisted of 125 nM purine and 6.25 nM HP-921 MS tuning mix (Agilent Technologies, Waldbronn, Germany) in methanol/water (90/10, v/v) for continuous mass calibration. The flow rate was 0.05 mL/min (Table S- 8, p. 193, isocratic pump 3) for RPLC-HILIC analyses and 0.2 mL/min for SFC analyses (Table S- 9, p. 193, isocratic pump). All samples were analyzed in positive and negative ESI mode. Jet-Stream ESI-source parameters used for both chromatographic systems are shown in Table S- 10 (p. 194). The resolution of the instrument was better than 10,000 at m/z 922.

6.2.5 Evaluation of chromatographic reproducibility

To assess the reproducibility and stability of both chromatographic techniques, all standard compounds were injected three times at the same day and three times within one week. The acquired retention times were used to consequently determine the intraday and interday stability. For standard compounds, which were only detected in positive or negative ESI mode, three RTs were used for the calculation of average RT and relative standard deviation (RSD) of RT. For compounds, which were detected in positive and negative ESI mode, all six RTs were considered. The mean retention times of intra- and interday analyses were applied for the screening results in the exemplary wastewater treatment plant effluent sample.

6.2.6 Solid phase extraction (SPE) of trace organic compounds from wastewater treatment plant effluent samples

The effluent of a large wastewater treatment plant in Bavaria, Germany, was sampled three times within one hour. As part of sample preparation for analysis, (trace) organic substances were extracted and concentrated using a two-stage solid phase extraction protocol, intended to retain very polar, polar, and non-polar compounds. Therefore, C18-SPE cartridges (Phenomenex Strata C18-E, Aschaffenburg, Germany) and ZIC-HILIC SPE cartridges (di2chrom, Marl, Germany) were used. Prior to loading on the ZIC-HILIC cartridges, the water sample was freeze-dried (Alpha 1-4, Christ, Osterode am Harz, Germany) and reconstituted in acetonitrile/water (95/5, v/v). The final sample contained acetonitrile/water (50/50, v/v) as solvent. The three processed samples were combined to a mix sample, containing equal volumes of each individual sample. This extraction was also performed on pure water (LC-MS grade) that served as a blank sample for further investigations. The SPE enrichment resulted in a concentration factor of 300 (volume of the final SPE concentrate in relation to the original sample volume). All samples were filtered through PVDF syringe filter units (13 mm, 0.22 µm, Berrytec, Gruenwald, Germany) prior to injection.

6.2.7 Determination of matrix effect

To evaluate MS signal enhancement or suppression resulting from matrix effect, MS signal areas of analyses in samples with strong and weak matrix were compared. Therefore, 10 reference compounds, previously not detected in the sample, were added to the effluent sample in final concentrations of 10 µM. The sample was injected three times in both systems and each ESI-ionization mode. Signal areas of these measurements were compared to those of the same compounds in acetonitrile/water (50/50, v/v). Differences between strong and weak matrix signals were derived by two-sample t-test.

6.2.8 Data processing

Mass spectrometric data was acquired with MassHunter Workstation LC/MS Data Acquisition software (version B05.01) and processed with MassHunter Workstation Profinder software (version B06.00) (both Agilent Technologies, Waldbronn, Germany). Data evaluation was conducted on the basis of compound features, summarizing all adducts of a compound after peak picking. Reference standard RTs were derived by a threefold injection of the working standard mixtures. The enriched surface water sample data sets were analyzed for compounds with identical RT–mass pairs compared to standard compound analyses. Mass accuracy was expected to be closer than 10 ppm, and relative standard deviation of RT was expected to be in a range of 2% to 5% compared to the working standard mixture RTs. All substance specific properties were derived from the compound database STOFF-IDENT(STOFF-IDENT, 2017), also accessible through the open-access software platform FOR-IDENT(FOR-IDENT, 2017). The theoretical compound polarity therein was originally processed via the pH-dependent logarithmic octanol-water distribution coefficient (log D) for each compound at pH 5 and 7 (ChemAxon, Budapest, Hungary).

6.3 Results and discussion

6.3.1 Reference standards to capture the polarity extension

Two chromatographic systems were evaluated for their applicability in screening strategies that involve the separation and detection of multiple organic compounds. The standard compounds used were chosen for their potential environmental relevance, intended use, and polarity. Detailed information was obtained from the open-access database STOFF-IDENT (STOFF-IDENT, 2017) as part of open access software platform FOR-IDENT (FOR-IDENT, 2017). This database currently contains information and physico-chemical properties for approximately 10,500 anthropogenic organic compounds (expected to occur in an aqueous environment) (Huckele and Track, 2013). All of the 274 compounds applied in this study were contained in STOFF-IDENT and mostly categorized into anthropogenic pharmaceutical active compounds, pesticides, personal care products, and biogenic compounds that originate from natural organic matter (NOM). Transformation products of several compounds were also included (Table S- 7, p. 179). Overall log D (pH 7) values in this study ranged from -7.71 to +7.67 and were calculated. The lower polarity limit of polar compounds was set to a log D (pH 7) value of -2.5 because compounds above this polarity can likely be retained by polar modified RPLC columns (Carr et al., 2015). The polarity region below a log D of -2.5 was mostly restricted to HILIC, NPLC, ion chromatography, capillary electrophoresis and other separation techniques for very polar molecules.

A total of 274 reference standards were applied in this study. 52 of the investigated compounds were very polar (log D <-2.5), 161 compounds were polar (-2.5 < log D <+2) and 61 were non-polar (log D >+2). Additional details are summarized in Table S- 7 on page 179.

6.3.2 Analytical systems applied for the polarity extension.

6.3.2.1 RPLC-HILIC

Two orthogonal chromatographic methods are combined in this analytical system as a serial coupling of RPLC and HILIC (Horváth et al., 1976; Greco et al., 2012). This setup provides advantages of both techniques, namely the retention and separation of polar and non-polar by RPLC as well as the retention and separation of polar and very polar molecules by HILIC. Both techniques can be handled with mobile phases, consisting of acetonitrile and water. The positioning of the RPLC column ahead of the HILIC column in this serial coupling preserves all of the features of RPLC separation. The injection of aqueous samples and retention order of molecules remain the same as that for “classical” RPLC. The connection of the two columns through a T-piece and a second binary pump for the additional mobile phase flow rate and composition provides the ability to change the mobile phase composition before entering the HILIC column from high aqueous content to high organic content. The remaining proportion of water in the mobile phase ensures the integrity of the thin water layer on the stationary phase, which is critical for successful HILIC separations (even when injecting aqueous samples) (Greco et al., 2012). Consequently, polar and very polar compounds that are not retained on the RPLC column can be retained on the HILIC column. Compound separation and elution begins in the HILIC column and is followed by the RP column. The compounds eluted in two clusters that represent HILIC- (left cluster) and RPLC-retained (right cluster) molecules (Figure 6-2a and Figure S-1) (Rajab et al., 2013). The right cluster contains all the molecules that would also have been separated and detected by single column RPLC-MS. The transition time from HILIC-retained compounds to RPLC-retained molecules is

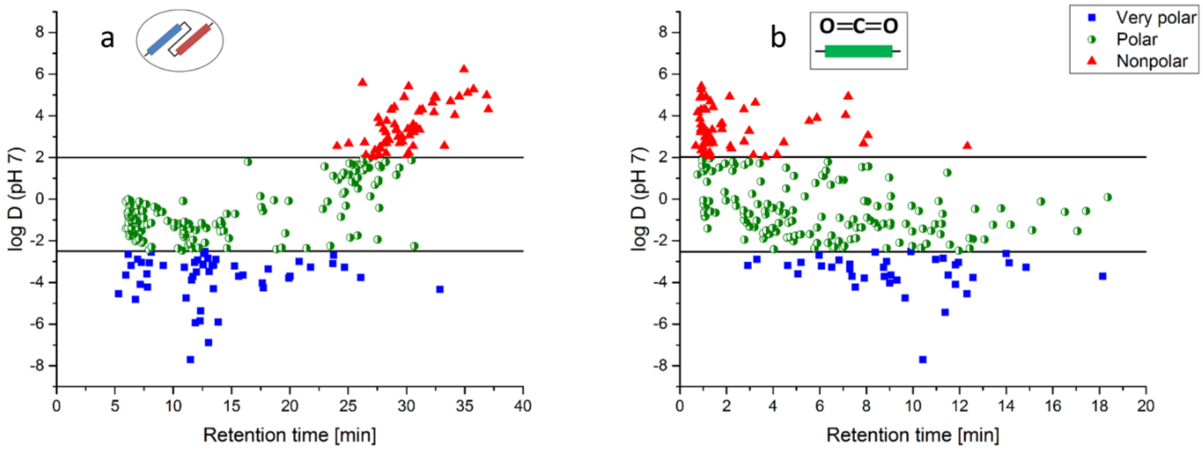

Figure 6-2: RT – log D (pH 7) plots of standard compounds analyzed by RPLC-HILIC/TOF-MS (a) and SFC/TOF-MS (b). Very polar compounds (log D (pH 7) <-2.5, blue rectangles) are mainly retained by HILIC in the RPLC-HILIC coupling, while non-polar compounds (log D (pH 7) > 2.0, red triangles) are exclusively retained by RPLC. Polar compounds are retained in both, HILIC and RPLC, but retention in HILIC seems to be more likely with increased polarity. In SFC (b), non-polar compounds are retained less than very polar compounds. The retention patterns in the RPLC-HILIC coupling (a) shows two groups that represent HILIC- (RT < 16 minutes) and RPLC- retained compounds (RT > 16 minutes). Compound log D increases with RT in RPLC, while the opposing occurs in HILIC. This normal-phase comparable retention behavior can partly also be observed in SFC separations (b).

approximately 16 minutes RT. The polarity of compounds eluting in the first 16 minutes increases with increasing water content in the HILIC separation gradient (log D values decrease). The polarity after 16 minutes of eluting compounds decreases with increasing acetonitrile content in the RPLC gradient (log D values increase). A study of RPLC-retarded molecules found some of them to be very polar as well as polar and non-polar and RTs of polar and non-polar compounds tended to increase systematically with an increasing log D value (Figure 6-2a triangles and circles). The RPLC column affected retention for some very polar compounds with log D value as low as -4.33, but for the majority of polar and very polar compounds, HILIC separations are more suitable (Periat et al., 2013). All compounds retarded on the HILIC column possessed exclusively negative log D values. However, a linear correlation between RTs on HILIC and compound log D/polarity could not be observed. The main reason is that there are more complex retention mechanisms in HILIC that exhibit ionic exchange and electrostatic interactions in addition to partition mechanisms (Greco et al., 2012; Kumar et al., 2013). Compound polarity as well as the charge state contributes to compound retention behavior in HILIC as a result. The HILIC method with a zwitterionic stationary phase fits for satisfying chromatographic conditions (as shown in Figure S-4). The properties for single molecule separations can be optimized respectively to eliminate peak broadening as well as peak tailing (e.g. salt content or pH) like published previously for this stationary phase type (Greco et al., 2012; Greco and Letzel, 2013). However, due to a generic separation required for screening techniques, no focus was placed on individual optimal conditions. Neither this was done for RPLC and the SFC screening conditions.

6.3.2.2 SFC

SFC separations are considered comparable to normal phase LC separations (Taylor, 2008) and injections of aqueous samples in a SFC systems are possible (Taylor, 2009). Non-polar compounds should elute earlier than polar compounds as a consequence. To visualize the relation of compound polarity and retention behavior, the log D at pH 7 was plotted as function of the RT (Figure 6-2b). A correlation of RT and compound log D/polarity, could not be verified for SFC separations. Same was true for log D values at pH 5, which is closer to the real pH of here applied SFC separations (Figure S-2) (Lesellier and West, 2015). Although most non-polar compounds eluted early, some have high RTs (eluting between 4 and 12 minutes). Comparable trends are observed for polar and very polar compounds. In contrast to RPLC-HILIC separations, compound polarity does not seem to be an appropriate indicator for RT estimations. This study used the same stationary phase type for both SFC and HILIC (in the RPLC-HILIC coupling) that possessed zwitterionic characteristics. However, the elution order of the polar compounds was not the same in both. Polar/ionic interactions between analytes and the stationary phase in addition to hydrogen bonding abilities (West et al., 2012) likely play a different role in the SFC and HILIC retention mechanisms. The presence of ionic interactions in SFC separations has already been identified in pentafluorophenyl stationary phases (West et al., 2015). The unique characteristics of CO_2 in SFC may also contribute to the retention, and lead to compound retention behavior not comparable to LC observations. The polarity range of separable standard compounds in SFC was the same as that obtained with the RPLC-HILIC. This is remarkable, because SFC separation was performed using only one hydrophilic column instead of two (hydrophilic and hydrophobic) coupled columns. Although certain compounds eluted early from SFC (red triangles Figure 2b), these compounds have significant retention (see k values in Table S- 12 on page 206), valid chromatographic peak shapes, specific masses and are MS detectable. Please note, a high amount of closely eluting or co-eluting compounds may result in decreased sensitivity in MS detection due to signal suppression effects by sample matrix.

6.3.3 Chromatographic evaluation of the polarity extension.

RTs, RSDs, and ESI detection modes from this section are summarized in Table S- 11 (p. 195) (RPLC-HILIC/TOF-MS) and Table S- 12 (p. 206) (SFC/TOF-MS).

6.3.3.1 RPLC-HILIC

262 reference standards (out of the 274) were retained, separated, and detected by RPLC-HILIC/TOF-MS. 132 standard compounds were exclusively detected in positive ESI mode and 23 in negative ESI mode. 107 compounds were detected in both ionization modes. The RSDs of RTs using RPLC-HILIC in three interday injections were less than 2% relative standard deviation (RSD) for all compounds. The

reproducibility of intraday experiments was as good as for interday measurement, except for metformin which showed a RSD of 2.9%. The overall RSDs of inter- and intraday were below 2% for 258 of 262 of all standard compounds and even lower than 0.2% for 102 standards (Figure 6-3a). Only the overall RSDs of metformin (RSD =3.5%), 2-pyrrolidin-1-ylethanol (RSD =2.2%), 2-(dimethylamino)-2-methylpropan-1-ol (RSD =2.1%), and diatrizoate (RSD =3.0%) were higher. So, the observed RSDs were in the same range as previously reported for this technique (Greco et al., 2013). To ensure a sufficient equilibration for HILIC separations a re-equilibration of the stationary phase with 15 to 20 column volumes is advised (Greco and Letzel, 2013). The method used in this study contained a re-equilibration phase with 49 column volumes. To ensure this re-equilibration period was sufficiently long enough for the RPLC-HILIC coupling, a subset of 29 HILIC retained and 25 RP retained compounds was exemplary separated by RPLC-HILIC after an extended equilibration period of 109 column volumes. The acquired RTs deviated from those with shorter re-equilibration by less than 2% (Table S- 11 on page 195). This demonstrates the equilibration phase with 49 column volumes results in a highly reproducible and complete equilibration state.

In total 126 of the 262 standard compounds (48.1%) eluted in this study from the RP column and 136 (51.9%) from HILIC. While non-polar compounds were exclusively retained in the RP column (60 of 60 non-polar standard compounds), only 36% of the polar compounds (54 of 151 polar standard compounds) and 23.5% of the very polar compounds (12 of 51) could be separated by RPLC. As a consequence, 64% of all polar and 76.5% of all very polar were only separable on the HILIC column in the serial coupling. The retention of polar and very polar compounds in RPLC might be further promotable to a certain degree by utilizing of polar modified or polar embedded stationary phases. However, many especially very polar compounds might not be retained in RPLC, for which HILIC (or SFC) is a suitable alternative (Periat et al., 2013).

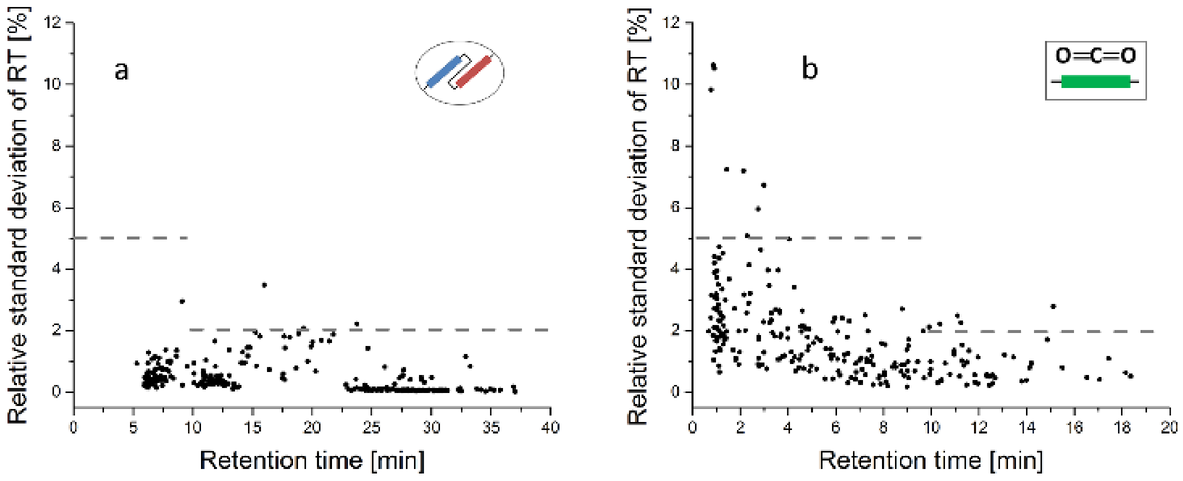

Figure 6-3: Overall relative standard deviations of overall retention times from intra- and interday analyses of standard compounds by RPLC-HILIC/TOF-MS (a) and SFC/TOF-MS (b). For the following hidden target screening, 5% RSD were set as maximum for RTs below 10 minutes and 2% for RTs higher than 10 minutes.

6.3.3.2 SFC

245 of the 274 standard compounds were separable and detectable by SFC/TOF-MS. 227 of these compounds were detected exclusively in positive ESI mode, 18 compounds by both, negative and positive ESI mode and none exclusively in negative mode. The high number of compounds detected in positive ESI mode might be a result of the acidic pH of the mobile phase in SFC separations (Lesellier and West, 2015), which can promote the protonation of molecules in the electrospray ionization process. The separable compounds included 37 very polar, 151 polar and 57 non-polar compounds. The reproducibility of SFC separations was also investigated in inter- and intraday analyses. The overall RSDs of RTs were below 2% for 167 of 245 compounds. Higher RSDs were found for early eluting compounds, but for 237 compounds, RSDs were lower than 5% (Figure 6-3b). The equilibration of the stationary phase with five column volumes should be sufficient for reproducible separations (Taylor, 2009). To ensure a complete equilibration, 28 column volumes were used in this study.

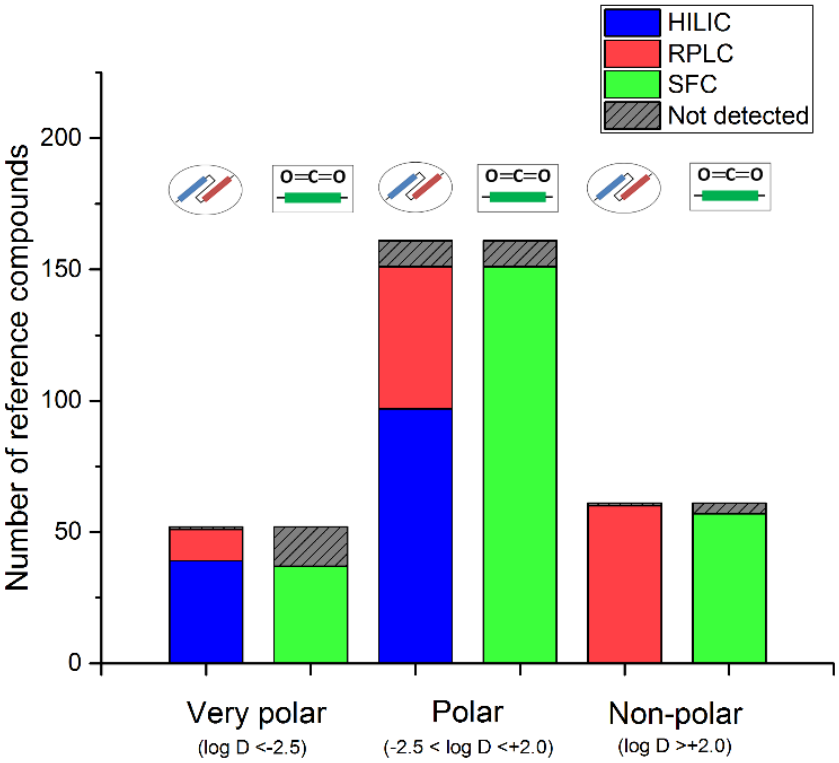

Figure 6-4: Number of standard compounds separated and detected by individual techniques. Blue sections indicate compounds which were separated by HILIC and, red by RPLC and green by SFC. Grey sections indicate compounds which were not detected by the according separation technique. Compounds which were not detected by RPLC-HILIC/TOF-MS were nonetheless detected by SFC/TOF-MS and vice versa.

The results of both separation techniques were complementary in matters of separable compounds (Figure 6-4). 15 very polar, 10 polar, and 4 non-polar compounds were only detected by RPLC-HILIC/TOF-MS and 1 very polar, 10 polar and 1 non-polar compound by SFC/TOF-MS. The detection of individual compounds might be promotable by altering ionization parameters of the ESI source, or adjusting the separation method.

6.3.4 Application of polarity extended chromatography with mass spectrometric detection for environmental water samples.

Screening (unknown) molecules in environmental samples such as wastewater treatment plant effluents or surface waters can be realized by target, suspects, and non-target screening techniques (Letzel et al., 2015). This study applied the hidden target strategy, using a database specially designed to contain the RTs and highly accurate masses of standard materials such as those referred to in Table S- 7 (p. 179). Hidden target screening detects molecules in a non-targeted type of measurement (i.e., the range of detected m/z from 50 to 3,200 with mass accuracy < 10 ppm) and uses information from the suspects screening type of data analysis (e.g. using analytical, chemical, or compound databases). The hidden target screening strategy was applied on samples of a wastewater treatment plant effluent. The analyses of treatment plant effluents is an increasingly important work, because wastewater treatment plants effluents are a significant entrance for trace organic compounds into the environment and provides epidemiological information (Eggen et al., 2014; Gracia-Lor et al., 2016).

Prior to analyses by polarity-extended separation techniques with highly accurate mass spectrometry, the compounds contained in the sample were enriched by SPE, one of the most commonly used pretreatment techniques (Richardson and Kimura, 2016). The SPE protocol utilized in this study was designed to enrich all compounds independent of polarity. C18 SPE was applied to enrich polar to non-polar compounds and HILIC SPE for very polar to polar compounds. The three pretreated samples were pooled afterwards, to gain a representative mixed sample. Equal amounts of the mixed sample extracts and the blank were injected three times in each ESI ionization mode into the individual analytical systems. Data from analyzed samples were processed to identify compounds using average RTs from inter- and intraday analyses and the accurate masses of reference standard compounds analyzed by RPLC-HILIC/TOF-MS and SFC/TOF-MS. The limits of RT deviations from RTs of standard compounds were 5% for RT < 10 minutes and 2% for RTs > 10 minutes. These limits were set according to the results of inter- and intraday stability experiments (Figure 6-3). Mass accuracy of detected compounds was expected to be better than 10 ppm and only MS signals with intensities of higher than 1x 10^3 counts were considered, which were absent in blank measurements. These restrictions were set in order to avoid mismatching of compounds with close eluting compounds with similar masses. Based on these restrictions, 79 of the standard compounds could be found and were identified in the real sample by accurate mass and RT (Table S- 13 on page 216). Among these were 14 very polar, 59 polar and 6 non-polar compounds.

RPLC-HILIC/TOF-MS detected a total of 58 compounds, containing 13 very polar, 42 polar, and 3 non-polar substances (Figure 6-5). All very polar compounds were retained by HILIC, as well as 35 of the 42 polar compounds. The remaining 7 polar and 3 non-polar compounds eluted from the RP column. Thus HILIC contributed strongly to the number of detectable compounds and allowed the extension of the detection window to polar and especially very polar compounds. The number of detectable compounds might be even increasable by using more sensitive (tandem-) MS or more intense sample pre-concentration.

By SFC/TOF-MS 42 of the standard compounds could be detected in the water sample, including 3 very polar, 35 polar and 4 non-polar compounds. The polarity range of these compounds was comparable to the range of RPLC-HILIC/TOF-MS. 2 of the very polar compounds, 18 of the polar and 1 of the non-polar compounds were also detected by RPLC-HILIC/TOF-MS (Figure 6-5). The detection of compounds by two orthogonal techniques, as those utilized in this study can improve results significantly and lead to a conformation of compound identity (Bieber et al., 2016b).

The complementarity of both techniques can be caused by signal suppression or enhancement, due to matrix effect (Niessen et al., 2006; Gosetti et al., 2010). To assess the extend matrix effect in both chromatographic techniques, compounds which were previously not included in the sample were added. Then, mass spectrometric signal areas of compounds in the sample were compared to signal areas derived from analyses of a reference standard mixture (Table S- 14 on page 220). In RPLC-HILIC/TOF-MS the real matrix caused signal suppression for 8 of the 10 compounds in positive ESI mode. In negative ESI mode, signal areas were lower for the four detectable compounds, compared to positive ESI mode but influence of matrix was minor (Figure S-3). The effects of matrix on ESI ionization in SFC/TOF-MS were not comparable to RPLC-HILIC/TOF-MS analyses. Some compounds suffering from signal suppression in RPLC-HILIC/TOF-MS in presence of matrix showed signal enhancement in SFC/TOF-MS measurements. None of the compounds were detected in negative ESI mode by SFC/TOF-MS. The mobile phase in SFC consists of compressed CO_2 and liquid modifier. The instantaneous expansion of the CO_2 after the backpressure regulator (Figure 6-1) supports the spray and the desolvation process. Furthermore, current high-flow MS inlets easily handle flow rates up to several mL/min without sensitivity loss. This may lead often to improved sensitivity for SFC-MS compared to LC-MS analyses (Grand-Guillaume Perrenoud et al., 2014). As a consequence, for quantitative analyses, a thorough assessment of matrix effect for all analytes is required independently.

6.4 Conclusions

Knowledge regarding very polar trace organic compounds is still scarce and monitoring these compounds is a challenge. Both techniques offer evidence to fill the current analytical gap by widening the perspective (not only in the environmental analysis). RPLC-HILIC/TOF-MS and SFC/TOF-MS provide highly reproducible separations, independent from compound polarity and absolute RT.

The separation mechanisms in RPLC-HILIC provide the option to gain information about compound polarity and even identity (in suspect and non-target screening) on the basis of RT. In addition, compounds eluted from HILIC can clearly be differentiated from those, eluted from RP. Although the SFC system utilized only one column, the same compounds like in the RPLC-HILIC serial coupling could be retained. Some compounds, mainly non-polar compounds have minimum retention in SFC, but can be detected with sufficient peak shape and specificity. In this case SFC provides the option to separate very polar to non-polar compounds in a smaller elution window, compared to RPLC-HILIC.

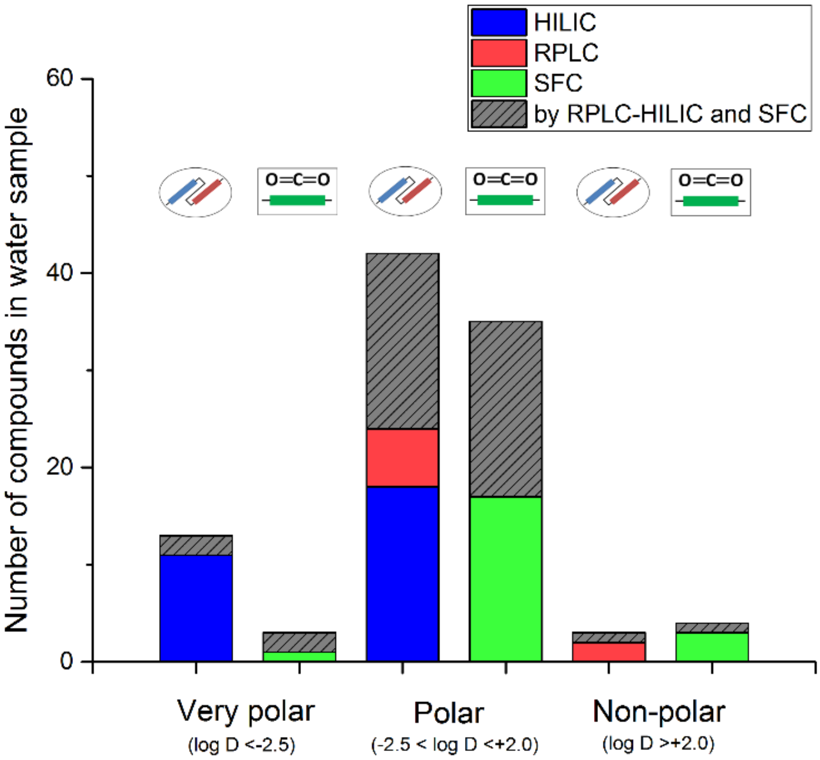

Figure 6-5: Standard compounds which were detected in the wastewater sample. Blue sections indicate compounds which were separated by HILIC, red by RPLC and green by SFC. Grey sections indicate compounds, which were separated by both, RPLC-HILIC/TOF-MS and SFC/TOF-MS.

Both techniques are applicable for hidden-target screening in environmental water samples and provide complementary results, offering the opportunity to separate and detect compounds, which are commonly not captured. Furthermore, the orthogonal separation techniques can be applied to validate each other. Complementary retention times (due to different retention mechanisms) and accurately detected masses (optional including MS/MS spectra) result in a new compound identification strategy.

The hypothesis that polarity extended separation techniques widen the view on TOrCs present in the aquatic environment, by detecting more polar environmentally relevant compounds in water samples than reversed phase LC-MS can be accepted.

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6.7 Supporting Information

The Supporting Information is provided in the Appendix of this thesis and available online at: http://pubs.acs.org/doi/suppl/10.1021/acs.analchem.7b00859/suppl_file/ac7b00859_si_001.pdf

Chapter 7 - Widening the analytical perspective – polarity extended separation for monitoring of trace organic compounds in surface water matrices[4]

Today monitoring trace organic compounds in water bodies is part of many strategies aiming to protect environmental health or drinking water quality. The occurrence of hazardous compounds in water bodies can be assessed using different analytical screening strategies. Reversed phase liquid chromatography (RPLC) coupled to mass spectrometric detection is a commonly used technique. RPLC is well suited for the separation and detection of medium to non-polar compounds, but can hardly be used for the detection of polar compounds. To cover the entire range from non-polar to very polar compounds in water bodies, a serial coupling of RPLC and hydrophilic interaction liquid chromatography (HILIC) or a supercritical fluid chromatography (SFC) system, both coupled to a time-of-flight mass spectrometer (TOF-MS) pose a new separation and detection technique. Both novel techniques were applied for target and suspects screening. The polarity range of the two techniques was comparable and covered the full range from non-polar (log D (pH 7) = +7.67) to very polar (log D (pH 7) = -7.86) properties. In addition to the extension of accessible polarity space for separations, the application of RPLC-HILIC/TOF-MS and SFC/TOF-MS in parallel improves the level of confidence in compound verification. For the verification of suspect compounds in water samples, the comparison of RPLC-HILIC/TOF-MS and SFC/TOF-MS data could substitute tandem-mass spectrometric data.

This publication tested the hypothesis that the complementarity of RPLC-HILIC/TOF-MS and SFC/TOF-MS improves the data quality in suspects- and hidden-target screening strategies, leading to improved TOrCs identification.

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[4] Bieber, S., Ruppe, S., Grosse, S., Drewes, J.E., Letzel, T., 2016. Widening the Analytical Perspective: Polarity Extended Separation for Monitoring of Trace Organic Compounds in Surface Water Matrices, in: Drewes, J.E., Letzel, T. (Eds.), Assessing Transformation Products of Chemicals by Non-Target and Suspect Screening − Strategies and Workflows Volume 1. American Chemical Society, Washington, DC, pp. 103–117. doi:10.1021/bk-2016-1241.ch007

Chapter 7 - Widening the analytical perspective – polarity extended separation for monitoring of trace organic compounds in surface water matrices

7.1 Introduction

The occurrence of chemical compounds in the environment has been well documented for many decades. In the 1960s, public concerns emerged that the presence of pesticides in the environment may cause adverse effects on environmental health (Carson, 1962). As a consequence, the environmental protection movement gained strong interest and legislative measures followed in many countries worldwide. For the protection of the environment and the selection regarding suitable protective management strategies, appropriate and sensitive environmental monitoring is required. While initially environmental monitoring was limited to a few compound groups mainly in use in industrial applications and agriculture, it became obvious quickly that many chemical compounds of daily use have the potential to enter the environment. Increasing knowledge about origin, source contributions and fate of compounds in the environment led to a strong increase of monitoring efforts, mainly relying on gas phase and liquid phase chromatography coupled to sensitive detectors, like mass spectrometry, to detect organic compounds (Schymanski et al., 2015). The huge diversity and broad polarity spectrum of detectable compounds in environmental water samples make it necessary to develop new analytical techniques. The emergence of polar transformation products from parent compounds generated during chemical or biological water treatment processes, emphasized the need to include new objectives in water quality monitoring (Boxall et al., 2004). In response to these needs, stationary phases in reversed phase liquid chromatography (RPLC) were modified with polar groups. Although such 'non endcapped', 'polar endcapped' or 'polar embedded' stationary phases have enhanced the RPLC separation power for polar compounds (logarithmic octanol-water distribution coefficient at pH 7, log D (pH 7) >-2.5), 'endcapped' RPLC remains more suitable for non-polar to medium polar compounds. Whereas hydrophilic interaction liquid chromatography (HILIC) would be more suitable for the separation of (very) polar compounds (Greco and Letzel, 2013). One option to combine the polarity ranges of separations is presented by a serial coupling of RPLC and HILIC representing two orthogonal separation techniques. This offers the separation of both, (very) polar and non-polar compounds in a single run (Greco et al., 2013). Besides LC techniques, supercritical fluid chromatography (SFC) can be used for polarity extended separations. The mobile phase of SFC separations is mainly comprised of carbon dioxide, which is considered a green solvent reducing the generation of less environmentally friendly solvent waste (Lesellier and West, 2015). Although SFC separations are generally regarded to be comparable to normal phase, the polarity range of SFC separations is significantly broader than common normal phase separations (Taylor, 2008; Desfontaine et al., 2015). The serial coupling of RPLC-HILIC and SFC have already been used in screening for trace organic compounds and can also be applied for non-target screening (Bieber et al., 2017). A combined approach using both techniques coupled with time-of-flight (TOF) MS detectors provides the benefit of increasing certainty in compound identification without necessarily utilizing tandem-mass spectrometry.

Target screening is a key element of environmental water quality monitoring. This approach is ideal for monitoring fully characterized, known compounds of environmental relevance. The concentration of target compounds in environmental water samples can be determined by using (isotope-labeled) internal reference substances (Letzel et al., 2015). In addition to these known compounds, unknown expected and/or unexpected compounds can be present in samples from aquatic environments. There is concern that some of the undetected compounds might also pose a risk to the aquatic environment or human health. As a consequence, additional and more sophisticated monitoring efforts are needed to gain information about the chemical universe contained in these water samples. Suspect and non-target screening strategies can be used to detect and identify such compounds at different levels of certainty (Schymanski et al., 2014; Letzel et al., 2015). Suspect screening utilizes different sources of information about the sample to identify possible compounds. As a basis for suspect screening serves a list of compounds, which might be detectable in environmental samples. In contrast to this compound-focused approach of suspect screening, non-target screening is based on mass spectrometric results (accurate mass and/or fragmentation patterns), which can be used to calculate the elemental composition of a compound and reveal chemical structure information. Furthermore, database matching can be used for the verification of compound identity, which corresponds to hidden-target screening (Letzel et al., 2015). A final confirmation of the compound identity is possible if a reference substance is available, raising non-target screening up to target screening level (Letzel et al., 2014, 2015; Schymanski et al., 2014).

7.2 Material and methods

Acetonitrile (ACN) and methanol, HiPerSolv Chromasolv LC-MS grade, were obtained from VWR (Darmstadt, Germany). Carbon dioxide (CO_2, purity 99.995%) was obtained from Westfalen AG (Muenster, Germany). Ammonium acetate was acquired from Sigma-Aldrich (Seelze, Germany). Isotope-labeled standards were obtained from Toronto Research Chemicals (Toronto, Canada), Neochema (Bodenheim, Germany), Dr. Ehrenstorfer (Augsburg, Germany), Sigma-Aldrich (Buchs, Switzerland), CDN Isotopes (Augsburg, Germany), EQ Laboratories (Augsburg, Germany) and ReseaChem (Burgdorf, Switzerland). The isotope-labeled standards were dissolved in different solvents and then transferred into four ethanol-based mix-solutions with concentrations ranging from 2.5 to 20 µg/mL. Standard compounds for standard suspect screening were purchased from Dr. Ehrenstorfer (Augsburg, Germany), Fluka (Buchs, Switzerland), Merck (Darmstadt, Germany), Parchem (New

Rochelle, New York, USA), Santa Cruz Biotechnology, Inc. (Dallas, Texas, USA) and Sigma-Aldrich (Seelze, Germany). Non-polar standard compounds were dissolved in acetonitrile, medium polar to (very) polar standard compounds in acetonitrile/water (50/50, v/v), resulting in stock solutions of 1 mM. Aliquots of the stock solutions were merged to working standard mixtures, containing 10 µM of each standard compound in acetonitrile/water (50/50, v/v).

The Rhine river water was obtained from the international monitoring station located in Weil am Rhein, Germany (river kilometer 171). The 24-h time-proportional composite sample was sampled and refrigerated (at 4°C) by automated samplers. The samples were extracted according to Kern et al. (Kern et al., 2009; Ruff et al., 2015) Briefly, the water samples were collected in glass bottles. 0.5 L of the sample was filtered through a glass fiber filter. The filtered samples were buffered using 0.5 mL 1M ammonium acetate and pH was adjusted to 6.5. A mix of isotope-labeled standards was then added to the samples. The samples were run through a packed solid phase cartridge. The cartridges were filled with 350 mg of a mixture of Strata-X AW, Strata-X CW (both Phenomenex, Aschaffenburg, Germany), ENV+ (Isolute, Biotage, Uppsala, Sweden) and 200 mg of Oasis HLB Material (Waters, Eschborn, Germany). After adsorption of compounds, the cartridges were set to dry for 30 minutes using a stream of nitrogen. The adsorbed substances were first eluted with 9 mL of ethyl acetate/methanol (50/50, v/v) + 2% ammonia and then with 3 mL of ethyl acetate/methanol (50/50, v/v) + 2% formic acid. The combined eluates were concentrated to 50 µL using a stream of nitrogen and then adjusted to 0.5 mL using purified water (Nanopure Diamond, Barnstead).

The serial coupling of RPLC and HILIC with mass spectrometric detection was utilized as previously reported (Greco et al., 2013; Rajab et al., 2013; Bieber et al., 2017). An Agilent 1260 HPLC system consisting of a degasser, a binary pump, an auto-sampler and a diode array detector was amended by a second binary pump (all Agilent Technologies, Waldbronn, Germany). The RPLC column (Poroshell 120 EC-C18, 50.0 × 3.0 mm, 2.7 µm; Agilent Technologies) was connected to the first binary pump, while the second binary pump was connected to the zwitterionic HILIC column (ZIC®-HILIC, 150 × 2.1 mm, 5 µm, 200 Å; Merck Sequant, Umea, Sweden). Both columns were connected through a T-piece (Upchurch, IDEX Europe GmbH, Erlangen, Germany). The system set-up is illustrated in Figure 7-1a. The mobile phase in the RP column consisted of 10 mM ammonium acetate in ACN/water (10/90, v/v) (solvent A) and 10 mM ammonium acetate in ACN/water (90/10, v/v) (solvent B). The second binary pump, serving the HILIC column utilized ACN (solvent C) and water (solvent D). The injection volume was 10 µL. During sample injection, mobile phase composition was 100% solvent A in RP and 100% C was added to the mobile phase before entering the HILIC column. Elution of retained compounds was started in HILIC by increasing the content of solvent D from 0 to 40% within 7 minutes. Compounds from the RP column were eluted by subsequent increase of the content of solvent B in the mobile phase from 0 to 100% within 25 minutes. The mobile phase composition added by binary pump 2 (HILIC) remained unchanged during the elution of RP compounds. This prevented any further interactions of RP retained compounds with the stationary phase in HILIC. The chromatographic system was connected to an Agilent 6230 time-of-flight mass spectrometer (TOF-MS) with a Jet-Stream electrospray ionization (ESI) ion source (both Agilent Technologies, Santa Clara, CA, USA). An isocratic pump was additionally connected to the inlet of the ESI source, providing a make-up flow for internal mass calibration.

The analytical SFC system (Figure 7-1b) consisted of a degasser, a binary pump, an auto-sampler, a temperature controlled column compartment, a diode array detector and a backpressure regulator (all Agilent Technologies, Waldbronn, Germany). A zwitterionic HILIC column (150 x 2.0 mm, 5 µm, Knauer, Berlin, Germany) was utilized for SFC separations. The mobile phase consisted of CO_2 and 20 mM ammonium acetate in methanol (modifier). The initial condition of the mobile phase was 5% modifier in CO_2, which was held constant for two minutes. Modifier proportion was subsequently increased to 40% within 13 minutes, kept constant for two minutes and reduced to initial conditions within one minute. The flow rate was set to 1.5 mL/min at a backpressure of 150 bar, a column temperature of 40°C and a 10 µL injection loop. The outlet of the SFC was connected to the ESI source of the TOF-MS, as described above for RPLC-HILIC, including the make-up flow. ESI parameters of RPLC-HILIC/TOF-MS and SFC/TOF-MS were applied as shown in Table 7-1 and described elsewhere (Bieber et al., 2017).

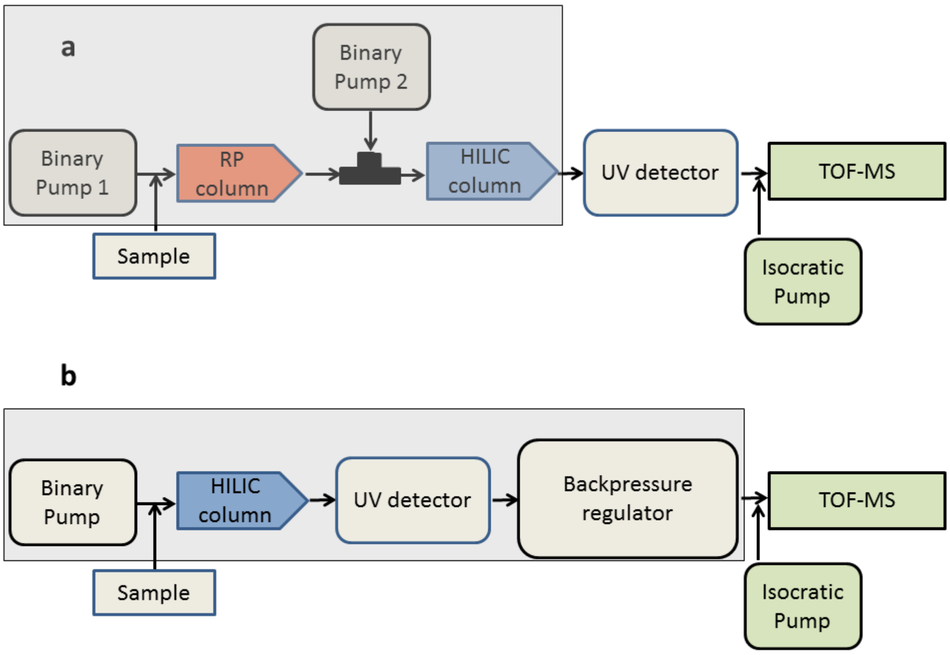

Figure 7-1: Scheme of the serial RPLC-HILIC coupling (a,). Both columns are connected via T-piece and two high pressure binary pumps are required to maintain optimal mobile phase conditions. The set-up of the SFC system (b,) is comparable to LC systems, but the high pressure binary pump, the column and the UV detector are pressurized in SFC separations. Grey regions represent the pressurized parts. Both systems were connected to a time-of-flight mass spectrometer.

The data was processed using MassHunter Workstation Profinder software (Agilent Technologies, Waldbronn, Germany). Compound search in samples was performed on the basis of compound formula. Compounds were imported to databases, using Agilent MassHunter PCDL Manager (Agilent Technologies, Waldbronn, Germany). Mass deviations were expected to be lower than 20 ppm and retention times were expected to not shift more than 0.5 minutes, compared to standard compound measurements. All compound-specific data was obtained from the database STOFF-IDENT, which was designed as part of project RiSKWa funded by the German Federal Ministry of Education and Research (Huckele and Track, 2013; STOFF-IDENT, 2017). The environmental water sample was analyzed in triplicates using each technique. Retention times of the isotope labeled standards were used for the identification of the corresponding non-labeled compounds in the sample. Standard compounds were injected once and retention times and masses were used for the search of corresponding signals in the water sample.

Table 7-1: Parameters of the ESI source, applied for the ionization of compounds separated by RPLC-HILIC or SFC.

	Sheath gas temperature [°C]	**Sheath gas flow [L/min]**	**Gas temperature [°C]**	**Gas flow [L/min]**
RPLC-HILIC/TOF-MS	325	7.5	325	10
SFC/TOF-MS	275	6	275	5
	Nebulizer gas pressure [psi]	**Capillary voltage [kilo volts]**	**Fragmentor voltage [volts]**	
RPLC-HILIC/TOF-MS	45	-3	100	
SFC/TOF-MS	45	-4	100	

7.3 Results and discussion

The surface water sample investigated in this study was initially subject to target screening. For this reason 134 reference substances of targeted compounds were added to the sample as isotope-labeled internal standards (IL-ISTDs) prior to the sample pretreatment procedure. Of these standards, 55 IL-ISTDs were non-polar (log D (pH 7) >+1.5), 77 medium polar (log D (pH 7) from -2.5 to +1.5) and 2 very polar (log D (pH 7) < -2.5). The water sample was analyzed by RPLC-HILIC/TOF-MS and SFC/TOF-MS. The data was studied for masses of IL-ISTDs, which were present in the sample. Subsequently, data were searched for features of unlabeled counterpart of the IL-ISTDs with matching retention times.

Identified features were directly compared to extracted ion chromatograms of corresponding IL-ISTDs. As an example, masses of metoprolol, its transformation product metoprolol acid as well as venlafaxine and its transformation product desvenlafaxine were detected in the surface water sample by RPLC-HILIC/TOF-MS (Figure 7-2a). Retention times were matching those of the corresponding IL-ISTDs present in the sample (Figure 7-2b). The chromatographic setup of the RPLC-HILIC technique helped to evaluate the polarity range of individual separations. Due to the chromatographic setup, HILIC retained compounds eluted before RPLC retained ones. The transition from HILIC to RPLC could be observed at a retention time of 15 minutes. The two transformation products were more polar than the parent compound and eluting earlier during RPLC-HILIC separation. Metoprolol acid was retained by the HILIC column, which means that the compound was not retained in the RP column and only detectable due to the polarity extension of RPLC by HILIC. The same four compounds could also be detected by SFC/TOF-MS (Figure 7-3). Retention order of compounds during SFC separation was not comparable to RPLC-HILIC analyses, due to different retention mechanisms. For a final verification of

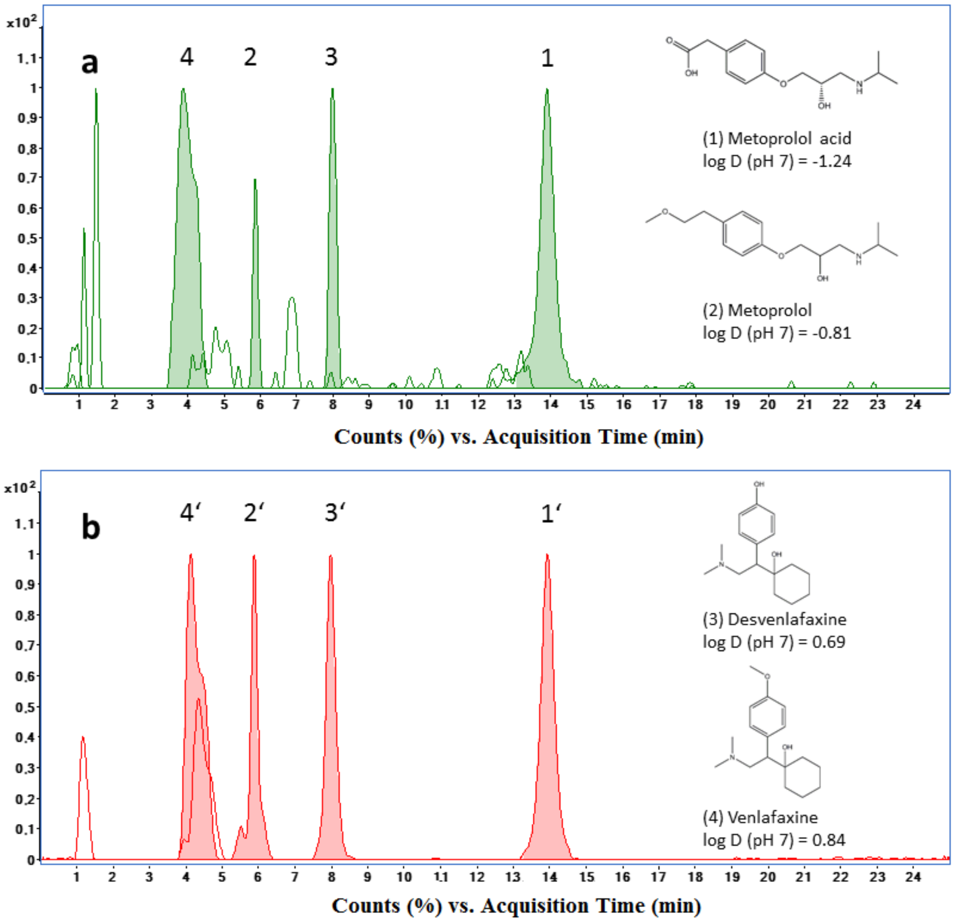

Figure 7-2: Extracted ion chromatograms (EICs) of metoprolol acid (1), metoprolol (2), desvenlafaxine (3) and venlafaxine (4), (a) found in the surface water sample, separated and detected by RPLC-HILIC/TOF-MS. All four compounds were previously being added to the sample as isotope-labelled internal standard compound and are shown as EICs in (b) with ‘.

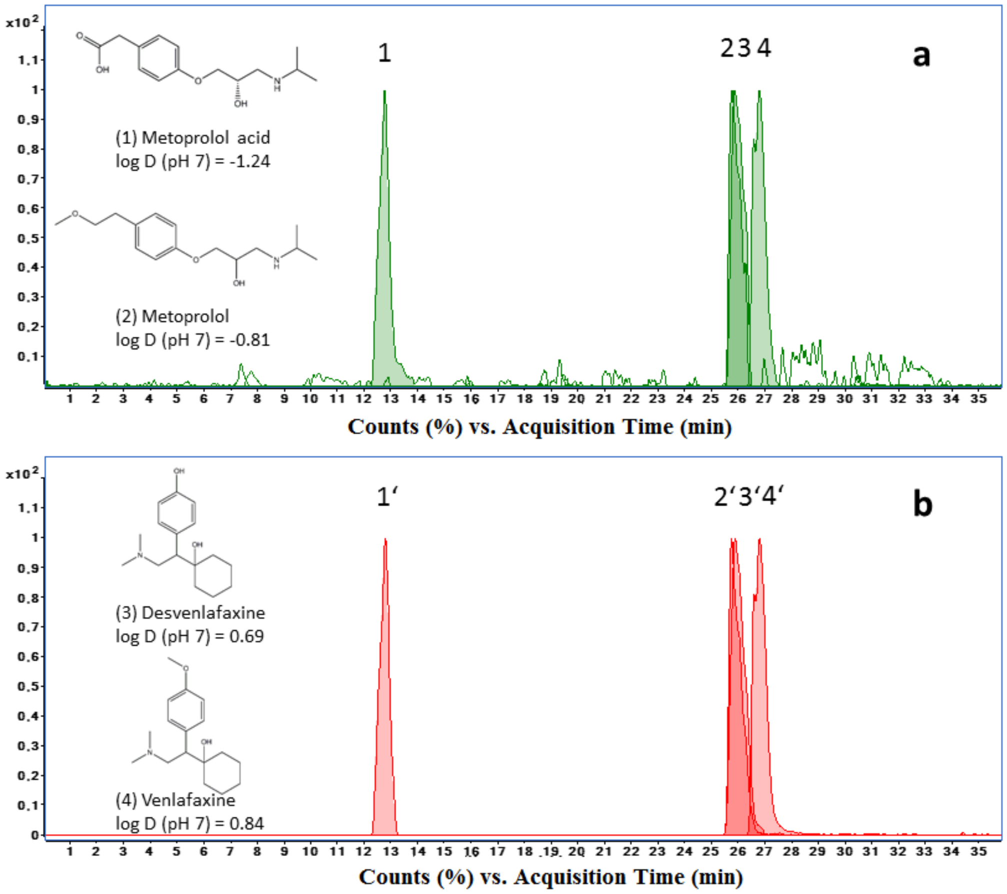

Figure 7-3: Extracted ion chromatograms (EICs) of metoprolol acid (1), metoprolol (2), desvenlafaxine (3) and venlafaxine (4), (a) found in the surface water sample, separated and detected by SFC/TOF-MS. All four compounds were previously being added to the sample as isotope-labelled internal standard compound and are shown as EICs in (b) with ‘.

compound identity, the comparison of compound fragmentation spectra and reference substance would be necessary. The TOF mass spectrometer, utilized in this study did not provide the option for MS/MS measurements. The application of RPLC-HILIC and SFC coupled with TOF-MS as two technologically independent separation techniques increased the credibility of obtained results significantly. As a result, the identity of detected compounds can be verified with a high degree of certainty, without the requirement to use tandem-mass spectrometric detection.

Suspects screening was conducted as a second step to identify compounds in addition to target compounds in the water sample. The basis of this screening approach was a list of compounds, which were suspected to be present in surface water samples. This list was derived from former non-target screening of various surface water samples. Features were typically extracted from the surface water data and compared to entries in the database STOFF-IDENT (STOFF-IDENT, 2017). Results from database matching were checked for plausibility and 152 of the proposed compounds were obtained as analytical standard. These compounds were mainly industrial chemicals, pharmaceuticals, pesticides, transformation products and compounds originating from natural organic matter. As a compound database, STOFF-IDENT exclusively contains water relevant molecules, which can be expected or have previously been detected in environmental aqueous samples (Huckele and Track, 2013). Database matching with STOFF-IDENT results in a lower number of matches for a feature, compared to mass spectrometric databases, but since all received hits account for molecules, expected in water samples, the quality of results is higher. In case of multiple database matches for one feature, compound information like polarity, compound usage or references from literature, which are contained in STOFF-IDENT, can be used to decide about the most suitable match. The polarity of the 152 compounds, investigated as suspect compounds in this study ranged from -7.86 log D (pH 7) to +7.67 log D (pH 7) All could be separated and detected by both, RPLC-HILIC/TOF-MS and SFC/TOF-MS. The attainable polarity ranges of RPLC can be visualized by a mass - retention time plot. In Figure 7-4a, the analyzed 152 suspect compounds (analytical standards) are plotted, using normalized retention times. The normalization took account of the transition from HILIC to RPLC after 15 minutes. This time was subtracted from all retention times. For compounds with retention times lower than 15 minutes, it was set to 1 minute. Using this normalization, data of the serial RPLC-HILIC coupling can be reduced to RPLC data, which could help to identify compounds previously detected by other RPLC methods (Abate-Pella et al., 2015). All non-polar compounds were exclusively retained by RPLC, while the number of medium polar and non-polar compounds retained by RPLC was limited. As described above, retention times in RPLC tended to increase with decreasing polarity. Most polar compounds showed no retention in RPLC. The addition of HILIC provides the opportunity to separate these RPLC non-retained compounds and widens the obtainable polarity range to very polar compounds (Figure 7-4b).

With only one chromatographic column, the SFC/TOF-MS system was also capable of separating and detecting the same compounds like the RPLC-HILIC/TOF-MS system. The attainable polarity range was identical to the serial LC-LC coupling. Elution of compounds occurred mainly from non-polar to polar and is similar to normal phase retention behavior (Figure 7-5) (Lesellier, 2009). Polar interaction, known to occur in HILIC stationary phases (Greco and Letzel, 2013) can lead to altered retention behavior for certain compounds, which might explain the high retention of some non-polar compounds and the low retention of certain polar compounds. The occurrence of polar interactions has previously been described for SFC separations (West et al., 2012).

Masses and retention times of the standard compounds, separated and detected by both techniques, were summarized in an in-house database. The surface water sample was analyzed with both polarity extended separation techniques, using the same separation and detection setting, as utilized for the standard suspect analyses.

In order to detect emerging or unknown compounds, data of the environmental sample was compared with data from standard compound analyses. Given the vast number of compounds, which can be

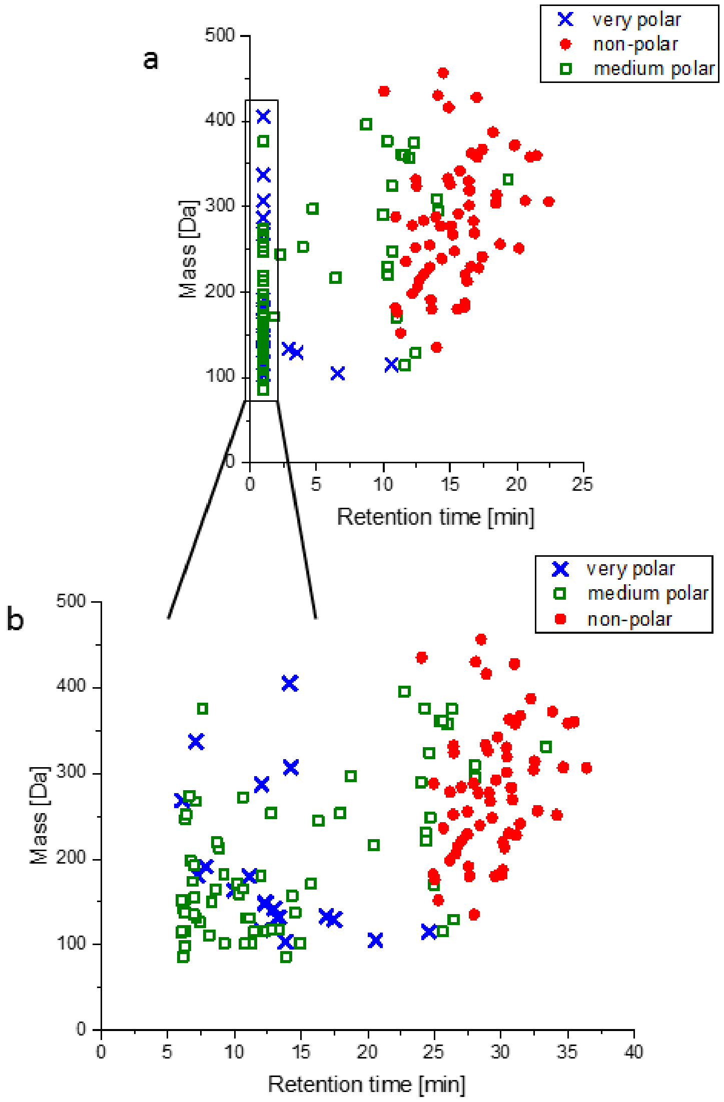

Figure 7-4: Mass-retention time plots of 152 standard compounds, separated by RPLC-HILIC/TOF-MS, grouped by compound polarity (very polar: log D (pH 7) <-2.5; medium polar: log D (pH 7) from -2.5 to +1.5; non-polar: log D (pH 7) >+1.5). Normalized retention times indicated the retention behavior of RPLC (a,). The serial coupling of RPLC and HILIC (b,) opens the polarity range of separations to more polar molecules.

present in environmental water samples, acceptable ranges for accuracy and retention times should be set strictly. This prevents false positive detection of isomeric or similar compounds contained in the sample. This topic requires even more consideration, since the number of detectable features is significantly higher when using polarity extended separations, compared to commonly used RPLC-MS analyses, due to additionally detected very polar compounds. However, database matching of features is not sufficient to verify compound identity in an environmental sample. In Figure 7-6, extracted ion chromatograms of isoniazid, the bisoprolol transformation product des(isopropoxyethyl) bisoprolol acid, melamine, the transformation product of metformin N-guanylurea (all polar) and tris(2-chloroisopropyl)phosphate (TCPP) (non-polar) are shown. All were obtained from suspect standard compound analyses (b). Comparable features for all compounds were found in the surface water sample by RPLC-HILIC/TOF-MS analyses (Figure 7-6a). Without the application of MS/MS detection this data would not be reliable enough to identify the features as the suspected compounds. The comparison of RPLC-HILIC/TOF-MS and SFC/TOF-MS data offers the opportunity to increase knowledge about the identity of a feature. As shown in Figure 7-7, features of the above mentioned compounds were also found by SFC/TOF-MS analyses. If features are detected in the surface water sample by both techniques and additionally match with data of a standard compound, analyzed by both techniques, the probability of mistaking features for isomeric compounds is significantly decreased and the quality of results increased. Using only one of the two separation techniques, the level of compound identification confidence (Schymanski et al., 2014) would be rather low, since only a sum formula could be verified. Data comparison of both independent techniques increases the level of confidence to the full knowledge of compound identity. Although the application of both separation techniques expands the polarity range of detectable compounds, the application of MS/MS detection will remain a requirement for quantitative analysis, as conducted in target screening. The hyphenation of RPLC-HILIC or SFC with MS/MS detection will be the next step in method development. Even without the use of isotope-labeled reference compounds, both techniques can be used to provide strong evidence about the presence of compounds in environmental samples and SFC/TOF-MS is often less sensitive to matrix suppression (Bieber et al., 2017).

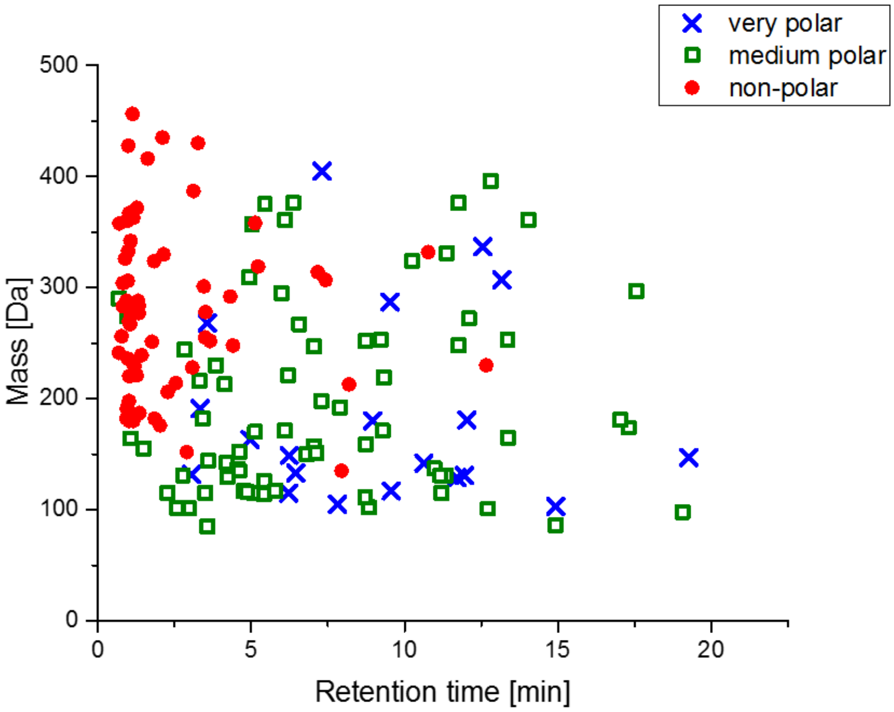

Figure 7-5: Mass-retention time plot of 152 standard compounds, separated by SFC, grouped by compound polarity (very polar: log D (pH 7) <-2.5; medium polar log D (pH 7) from -2.5 to +1.5; non-polar: log D (pH 7) >+1.5). The polarity range of SFC separations covered the full analyte spectrum.

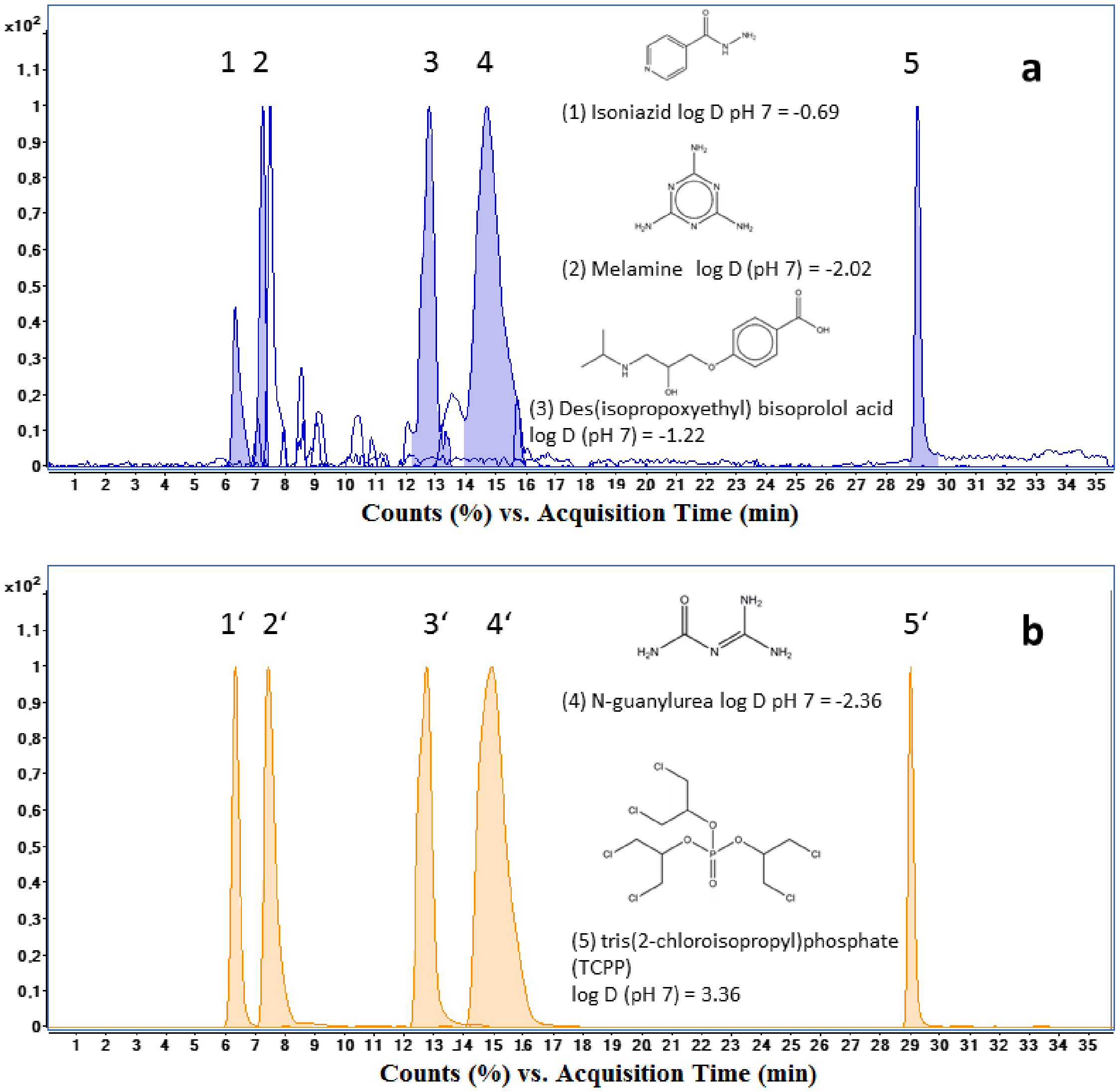

Figure 7-6: Extracted ion chromatograms of the surface water sample (a) of masses corresponding to isoniazid (1), melamine (2), des(isopropoxyethyl) bisoprolol acid (3), a transformation product of bisoprolol, N-guanylurea (4), a transformation product of metformine and TCPP (5) separated and detected by RPLC-HILIC/TOF-MS. All compounds were analyzed separately as suspect standard compound and are shown as EICs in (b) with '.

7.4 Conclusions

Target screening is and remains the key element of environmental water quality monitoring. Suspects screening using database tools via retention time and accurate mass can increase the accuracy of data evaluation. The application of RPLC-HILIC and SFC offers the opportunity to widen the range of separable and detectable compounds towards very polar compounds. With high robustness and reliability, RPLC-HILIC and SFC contribute to a better understanding of the presence of polar compounds in environmental water samples.

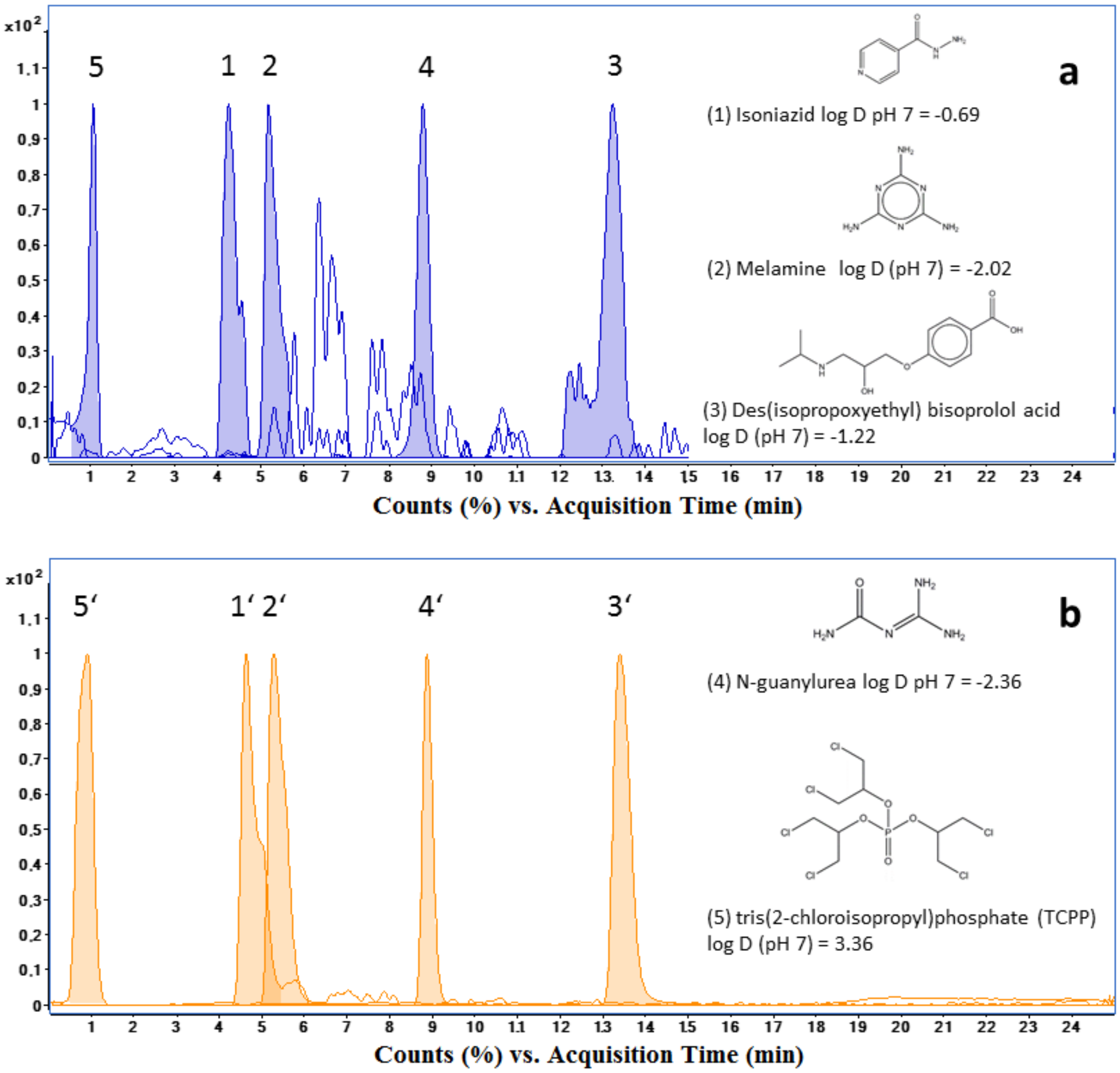

Figure 7-7: Extracted ion chromatograms of the surface water sample (a) of masses corresponding to isoniazid (1), melamine (2), des(isopropoxyethyl) bisoprolol acid (3), a transformation product of bisoprolol, N-guanylurea (4), a transformation product of metformine and TCPP (5) separated and detected by SFC/TOF-MS. All compounds were analyzed separately as suspect standard compound and are shown as EICs in (b) with '.

The application of both independent separation techniques resulted in more reliable results. The comparison of chromatographic results from RPLC-HILIC and SFC coupled with TOF-MS could be used as alternative approach for data validation if reference materials are available. This is due to the different chromatographic retention mechanisms for the same molecules. Consequently, this leads to a cross-evaluation by chromatography. Tandem mass spectrometry can further be used to evaluate the results from retention time and accurate mass, adding an additional dimension to data quality.

Using polarity extended chromatographic techniques like RPLC-HILIC and SFC allow retention, detection and identification of so far undetected compounds (by RPLC-MS). The broader separable polarity window is mandatory, when polar transformation products are targeted for the identification in environmental samples.

Ultimately, the combination of these techniques with tandem mass spectrometry will be an effective tool in water analysis. In the future, also unknown molecules will be classified with more information using the two polarity extended separation techniques.

The hypothesis that the complementarity of RPLC-HILIC/TOF-MS and SFC/TOF-MS improves the data quality in suspects- and hidden-target screening strategies, leading to improved TOrCs identification can be accepted.

7.5 Acknowledgements

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Chapter 8 - Discussion

The objectives of this dissertation were to evaluate management strategies for TOrCs in waterbodies to classify required analytical approaches and to investigate the benefits of the establishment of advanced analytical techniques for the management of TOrCs. Therefore, the thesis was centered on four research hypotheses which have been tested.

The first hypothesis investigated the underlying principles of management strategies for TOrCs. It was assumed that management strategies consisted of toxicity- and emission avoidance-based principles. Regardless of the principles for compound selection applied, analytical tools play a critical role in management strategies. Therefore, hypothesis #2 tested if toxicity- and/or emission avoidance-based strategies rely on the same analytical tools. Since RPLC-MS is most commonly used for the monitoring and/or identification of TOrCs, the analysis of very polar and polar compounds is a major challenge, but they might be highly relevant due to high mobility in the aqueous environment. To close this analytical gap and to gain a broader view on the universe of TOrCs, two advanced separation techniques, namely a serial RPLC-HILIC coupling and SFC were evaluated. Thus, the third hypothesis assumed that both separation techniques widen the range of detectable TOrCs in water samples towards very polar compounds. Subsequently, hypothesis #4 investigated if the parallel use of RPLC-HILIC/TOF-MS and SFC/TOF-MS can provide benefits for the screening for TOrCs in waterbodies. The following sections discuss the results of testing the hypotheses of this dissertation.

Hypothesis #1: Management strategies for TOrCs consist of toxicity- and/or emission avoidance-based principles

The investigated management strategies from the U.S., Australia, the EU and Switzerland exhibit little similarities at first glance. This already becomes obvious when comparing the motivations and drivers for the implementation of strategies and underlying geographical and economic conditions onto which the strategies were adjusted. The strategies reach from well-structured risk-management concepts for reused water (Australia), over water quality standards for toxicologically assessed compounds in drinking water and/or environmental waterbodies (U.S. and EU), to a precautionary-driven nation-wide initiative to upgrade wastewater treatment to reduce the discharge of TOrCs (Switzerland). Despite this heterogeneity regarding targeted health endpoints, drivers and paradigms, all implemented and proposed management approaches could be identified to follow either toxicity- or emission avoidance-based principles. Toxicity-based principles are followed in the U.S., Australia and the EU, management approaches based on emission avoidance were identified in the EU and Switzerland. Thus, hypothesis #1 was accepted.

Typically, toxicity-based approaches aim to assess the risk posed by the occurrence of individual TOrCs for a targeted endpoint. Setting water quality standards are a widely applied approach following this principle. These values are derived from toxicological assessment and implemented to prevent adverse effects on human or environmental health. The basis of quality standards can be effect or no-effect concentrations, combined with safety factors as a precautionary element. Thus, an exceedance of a water quality standard does not necessarily have to result in adverse effects for a protected endpoint, but can also trigger the implementation of mitigation measures (Dieter, 2009). Water quality standards or threshold values provide a quantifiable amount of safety against adverse effects from TOrCs, but consider only a limited set of compounds. Due to the high number of detectable compounds in waterbodies, a toxicological assessment of all compounds is not feasible. Compound specific water quality standards do not provide safety against adverse effects caused by mixtures of TOrCs, because these even can occur at concentrations below the no-effect concentration of single compounds (Fent et al., 2006). A toxicological assessment of compound mixtures is extremely challenging due to the high number of possible compound combinations and potential synergistic effects. For the assessment of mixture toxicity effects, modeling and predictive approaches might be more practical alternatives to classical toxicity testing (Brian et al., 2005; Escher et al., 2006). Such methods can also be applied for transformations products (TPs) and metabolites of known compounds, which otherwise have to be assessed separately (Escher and Fenner, 2011). However, by applying toxicity-based approaches, the uncertainty about potential risks posed by mixtures, TPs and unknown compounds cannot be ruled out. Another option is the definition of blanket values for groups of compounds. These values are not determined by specific toxicological data, but set so low that no adverse effects from exposure are assumed. In the EU, such values are used for pesticides with 0.1 µg/L for single pesticides and 0.5 µg/L for the sum of all pesticides (European Commission, 2006b). A comparable concept has been proposed by the Association of European Water Utilities, applicable for all TOrCs for which no other threshold values have been defined (IAWR et al., 2013).

In contrast to toxicity-based approaches, which accept the presence of TOrCs in waterbodies, as long as (scientifically derived) threshold values are not exceeded and no adverse effects are expected, emission avoidance-based approaches aim to prevent the release of TOrCs into waterbodies in general. The presence of any TOrC in waterbodies is regarded as potential risk for human and/or environmental health in general and should be prevented. This can for instance be achieved by the implementation of compound removal technologies at discharge points into the aquatic environment. For point sources like WWTP effluents, technological measures could be applied, removing (e.g. adsorption to activated carbon or membrane filtration) or degrading (e.g. ozonation) TOrCs leading to a significant reduction of compound emissions (Eggen et al., 2014). Anyhow, no advanced treatment technique is capable of removing all TOrCs. For non-point sources, the implementation of mitigation strategies is more challenging, because emission locations are not well defined. Here, organizational approaches like use restrictions for pesticides near waterbodies or compound substitution might provide a better impact on emission reduction than technological measures. A complete avoidance of compound emissions is hardly possible, because it would require a control of all entrance pathways for TOrCs into the aquatic environment. However, the application of such measures can result in a reduction of emissions for a broad range of compounds.

The combination of toxicity- and emission avoidance-based approaches offers the opportunity to combine the advantages of both principles and minimize potential short-comings. Toxicity-based approaches are compound specific and well-applicable for known and toxicologically assessable compounds. By accepting the presence of TOrCs in waterbodies, such approaches do only provide a limited degree of safety against potential adverse effects posed by unknown compounds, TPs or mixtures of compounds. These uncertainties can be reduced by aiming to prevent the emission of TOrCs, but compound emission avoidance measures are more difficult to implement. An example for a combination of both principles is the management strategy of the EU. General emission avoidance is applied to minimize TOrCs discharges into waterbodies, but knowing that this is not fully achievable, threshold values for known and toxicologically assessed compounds have been established. This combination provides safety against adverse effects posed by known compounds and reduces the risks posed by uncertainties like TP and compound mixtures. To determine the occurrence of known TOrCs and to identify unknown compounds, appropriate analytical tools are required to identify the wide range of different chemicals.

Hypothesis #2: Toxicity- and emission avoidance-based strategies use the same set of analytical tools to assess the efficiency of implemented measures and to monitor known and identify unknown compounds

The set of available analytical tools consists of target, suspects and non-target screening (Letzel, 2014; Letzel et al., 2015). While target screening is used for the quantification of known compound concentrations in samples, suspects and non-target screening can be used to identify compounds in samples. Management strategies with toxicity-based elements require target screening for the determination of concentrations of regulated TOrCs in waterbodies. In addition, suspects and non-target screening are needed to identify CECs, emerging contaminants and unknown compounds, which might pose risk to human or environmental health and could be candidates for toxicological assessment. In emission avoidance-based strategies, target screening can be applied to performance-based indicator compounds to monitor the efficacy of advanced water treatment processes or to determine compliance with blanket values. Qualitative screening strategies are used to identify emerging contaminants and TPs resulting from e.g. advanced water treatment techniques. As a result, toxicity- and emission avoidance-based strategies use the same set of analytical tools and hypothesis #2 can be accepted. Widening the window of analytical techniques would be beneficial for all management strategies, independent from underlying principles in compound selection. Great potential for improvements is provided by extending the polarity range of separation techniques used to identify and monitor TOrCs in aqueous samples.

Hypothesis #3: Polarity extended separation techniques widen the view on TOrCs present in the aquatic environment, by detecting more polar environmentally relevant compounds in water samples than reversed phase LC-MS

RPLC-MS(/MS) is well-suitable for the separation of polar to non-polar compounds, but for the separation of compounds with log D (pH 7) values below -2.5, alternative techniques are required (Periat et al., 2013; Reemtsma et al., 2016). The separation of more polar compounds could be achieved using NPLC or HILIC, but this requires a second analytical technique in parallel to RPLC. An ideal separation technique would allow to separate the same range of compounds as RPLC, but extend the polarity range towards more polar analytes. Thus, the serial coupling of RPLC and HILIC is a logical consequence, separating non-polar to polar compounds by RPLC and more polar ones by HILIC. The zwitter-ionic HILIC phase, which was used in the RPLC-HILIC coupling allows to separate very polar to polar uncharged (comparable to NPLC) and charged molecules (comparable to IC) (Greco et al., 2012). In the study conducted in this thesis, the RPLC-HILIC coupling was capable of separating compounds in a polarity range of -7.71 to +7.67, which is a significant extension compared to RPLC. It is worth mentioning that no compounds with lower and higher log D values were tested, so the full polarity range cannot be estimated yet. The same set of analytes could also be separated by SFC, which is remarkable, since the SFC separation was performed with only one stationary phase instead of two in the LC coupling. Both separation techniques exhibited high reproducibility and could successfully be coupled to an ESI TOF-MS. The application of the two systems for the detection of TOrCs in water samples was examined using pre-concentrated WWTP effluent (Chapter 6) and surface water samples (Chapter 7). In the WWTP effluent, the majority of detected compounds possessed a negative log D (pH7) and about 20% possessed a log D (pH 7) below -2.5. These compounds would have been hardly detectable by RPLC-MS. As a result, both separation techniques are capable of widening the analytical window of TOrCs and allow the detection of more polar compounds than RPLC. Thus, hypothesis #3 can be accepted. The complementarity of both separation techniques, which was observed for the analysis of standard compounds (Chapter 6), was further examined for suspects and hidden-target screening.

Hypothesis #4: The complementarity of RPLC-HILIC/TOF-MS and SFC/TOF-MS improves the data quality in suspects and hidden-target screening strategies, leading to improved TOrCs identification

The majority of the analyzed standard compounds (Chapter 6) could be separated by both techniques, but some compounds could only be detected by one or the other technique. This was also true for the analysis of the WWTP effluent sample. Thus, the application of both techniques in parallel allows to expand the set of detectable compounds. Matrix effect, which showed different impact on the ionization of analytes separated by RPLC-HILIC or SFC could be identified as one possible reason for this complementarity. Another benefit for suspects and hidden-target screening is provided in the set-up of the serial RPLC-HILIC coupling and the resulting correlation of RT and compound polarity (Rajab et al., 2013). It allows to estimate the polarity of unknown compounds depending on the RT, following the logic 'negative log D value for HILIC retained compounds' and 'increasing log D value for RP retained analytes'. In combination with open access tools, such as FOR-IDENT, RT-polarity correlations can help to identify compounds. Comparable information cannot be used for SFC separations so far, because retention mechanisms are not fully understood yet. However, LC comparable retention mechanisms were not observed for SFC separations, making SFC and LC orthogonal techniques (Parr et al., 2016). The degree of orthogonality between techniques has been visualized by plotting the RTs of standard compounds analyzed by both techniques against each other (data of Chapter 7). The diagram reveals that retention mechanisms of both techniques are not correlated, resulting in a high degree of orthogonality (Figure 8-1) especially for early eluting compounds in HILIC, which are well-separated by SFC and early eluting compounds in SFC, which are well-separated by RP. The combination of these orthogonal techniques allows to improve the quality of results in suspects and hidden-target screening due to the cross-validation of detected compounds.

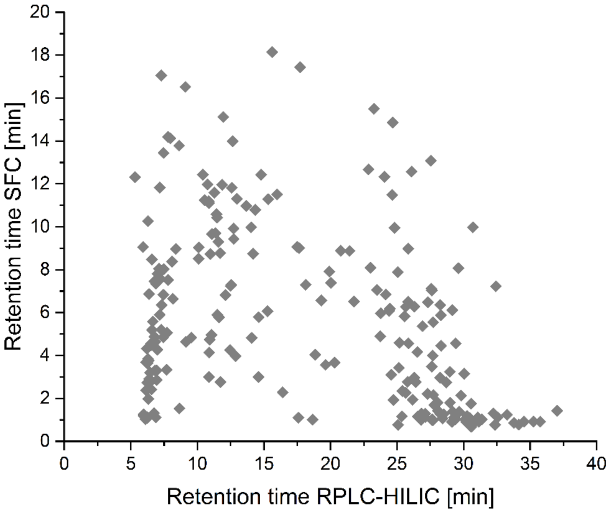

Figure 8-1: Graphical evaluation of the orthogonality of RPLC-HILIC and SFC. RTs of standard compounds, which were analyzed in order to evaluate the polarity ranges of the two techniques (Chapter 7) were plotted against each other. No linear correlation of RTs could be verified and the diagram reveals a high degree of orthogonality between both techniques. . Compounds hardly retained in HILIC are well-separable by SFC and compounds hardly separated in SFC are well-retained in RPLC.

Chapter 9 - Conclusions, additional research needs and future perspective

The establishment of holistic management strategies for TOrCs is extremely challenging, due to the high number of compounds which can enter the aquatic environment and their various sources. In holistic management strategies, there is a strong relation between occurring TOrCs, implemented mitigation strategy and analytical techniques (Figure 9-1). The occurrence of TOrCs in waterbodies might trigger the implementation of mitigation strategies, which require appropriate analytical techniques for water quality monitoring and the identification of new and emerging compounds in waterbodies. These new compounds have to be included in already implemented mitigation strategies. As a consequence, advances in analytical techniques allowing to detect more (relevant) compounds and to adjust mitigation strategies can directly lead to an improvement of existing management strategies.

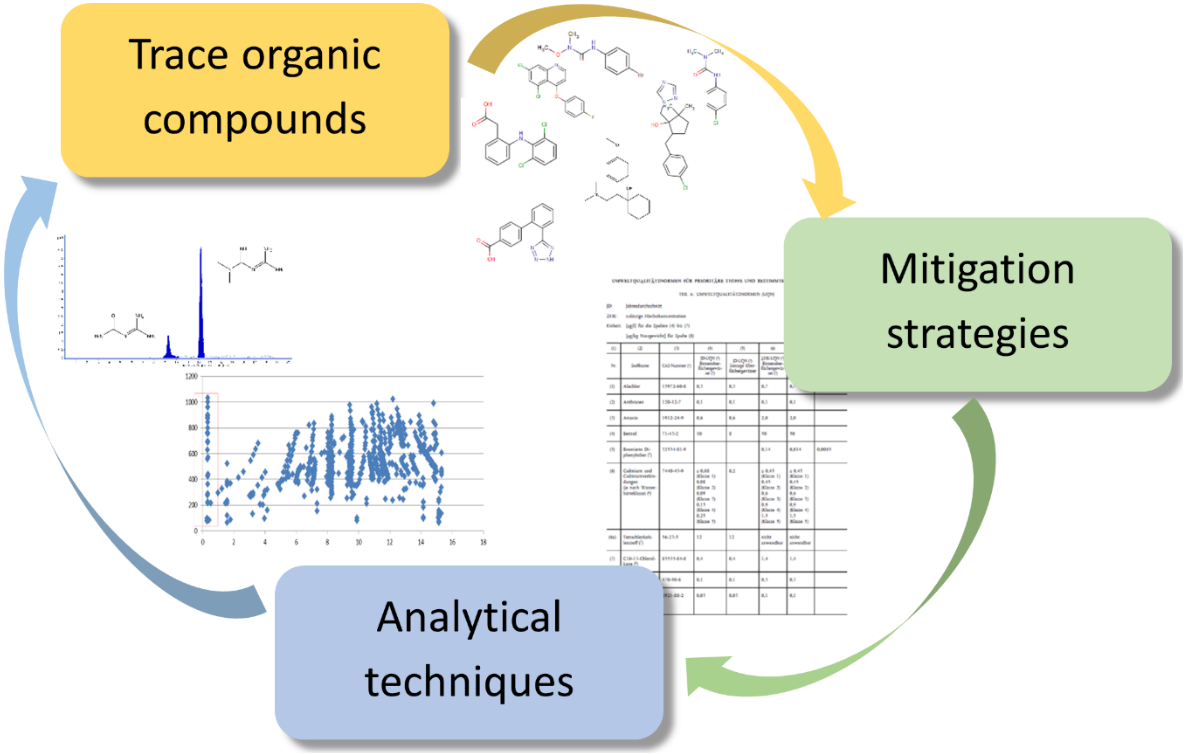

Figure 9-1: Optimal relations between TOrCs, mitigation strategies and analytical techniques. Comprehensive strategies aim to manage the universe of detectable and utilize analytical techniques for the monitoring and identification of TOrCs. Advanced analytical techniques allow the identification of new and emerging contaminants, which will have to be considered in mitigation strategies.

Strategies are implemented worldwide and follow comparable underlying principles. Toxicity-based approaches are suitable for the control of risks posed by known and toxicologically assessed compounds, but a compound by compound assessment of TOrCs is not feasible given the large number of detectable compounds. Water quality standards for individual compounds might not always be protective against mixture effects and unknown compounds. Emission avoidance-based approaches can reduce potential risks, posed by unknown compounds or mixtures of compounds, but a complete emission of TOrCs avoidance is hardly achievable. The combination of toxicity- and emission avoidance-based approaches appears as the most promising concept to control a wide range of compounds, covering known and hazardous TOrCs, mixtures and unassessed emerging contaminant or unknowns. For the implementation of such strategies, a comprehensive assessment of the life-cycle of chemicals is necessary, including registration, production, distribution, use and disposal of compounds. A major requirement for the establishment of holistic management strategies is represented by advanced water quality monitoring. This should include target-screening for known and assessed compounds and suspects and non-target screening for the identification and detection of CECs and emerging or unknown contaminants as well as their metabolites and TPs. Since not all of the constantly increasing number of detectable compounds pose a risk to environmental or human health, concepts to identify or prioritize relevant compounds are required. Thus, effect-directed analysis (EDA) will gain importance, because it combines analytical and toxicological tools for the identification and prioritization of health relevant TOrCs (Brack et al., 2016). In EDA, environmental samples are investigated for adverse effects using bioassays and if effects are observed, the complexity of the sample is stepwise reduced and fractions of the sample are tested again. Fractions which induce effects can ultimately be analyzed and chemical compounds which caused the effect could be identified. Advanced analytical separation and detection techniques are required to ensure that health relevant compounds can be identified successfully. Polarity extended separation techniques, such as RPLC-HILIC and SFC could be shown to provide benefits for the screening of TOrCs. Both techniques provide a more comprehensive view on TOrCs by widening the analytical window towards very polar compounds.

The differentiation between HILIC and RPLC separated compounds in RPLC-HILIC is a useful tool in suspects and non-target screening to estimate compound polarity. For RP separated compounds, RT can be normalized as retention time index (RTI) and used to calculate log D values. The application of RTI for suspects screening utilizing STOFF-IDENT has already been demonstrated previously (Schymanski et al., 2015). A comparable normalization approach for HILIC separated molecules is currently not available. However, computer-assisted method development in HILIC lead to reasonable results and can serve as starting point to create retention modeling and prediction tools, which could correlate RT and physico-chemical parameters of separated compounds (Tyteca et al., 2017). In SFC, further research is required to investigate basic retention mechanisms. A correlation of RT and compound polarity could not be verified, but depending on the interactions between analyte and stationary phase, further physico-chemical parameters might be brought in relation to RT. In a next step, the application of both investigated separation techniques for non-target screening has to be demonstrated. Considering the results of RPLC-HILIC and SFC for target and suspects screening, both techniques will certainly provide benefits for the identification of TOrCs by non-target screening. Both separation techniques now offer the chance to extend water analysis towards a completely new range of very polar compounds in water bodies. In concert with suitable data evaluation strategies and compound prioritization tools, these techniques can strongly contribute to an improved assessment of water quality. Ultimately, this will result in more holistic management strategies for TOrCs, considering the chemical universe present in the aquatic environment more comprehensively. This will result in an improved protection of the environment and drinking water resources and can contribute to reducing concerns and risks to environmental and human health by harmful trace organic chemicals.

Chapter 10 - Further publications and contributions

Peer reviewed journal articles:

Tyteca, E., Bieber, S., Letzel, T., Desmet, G., 2017. Possibilities and Limitations of Computer-Assisted Method Development in HILIC: A Case Study. Chromatographia 80, 771–781. doi:10.1007/s10337-016-3127-3128

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Bieber, S., Letzel, T., 2015. Superkritische Fluidchromatographie mit massenspektrometrischer Detektion (SFC-MS): Simultane Untersuchungsmethode für polare und unpolare organische Moleküle in Wasserproben. Gesellschaft Deutscher Chemiker, Mitteilungen der Fachgruppe Umweltchemie und Ökotoxikologie 2015, 1, 11–16.

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Contributions at conferences and workshops:

Oral presentations:

2014

Drewes, J. E., Bieber, S., Concepts and Principles to Manage CECs - German Perspective. Water Research Foundation #4494 Workshop 1a, Garching (D), 15th – 16th May 2014

Letzel, T., Bieber, S., Non-Target Screening, Suspected-Target Screening and Target Screening, Technologies and Philosophies, Databases and Crafts, Analytical Seminar - University of Arizona, Tucson (USA), 8th September 2014

Drewes, J. E., Bieber, S., International CEC Management Strategies – Lessons learned during WRF 4494 Workshop #1a, Water Research Foundation #4494 Workshop 1b, Dallas, Texas (USA), 10th – 11th September 2014

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Bieber, S., Greco, G., Letzel, T., Supercritical Fluid Chromatography (SFC) with Mass Spectrometric detection (SFC/MS): Pharmaceuticals in Water. SFC 2014, Basel (CH), 8th – 10th October 2014

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2015

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2016

Drewes, J. E., Bieber, S., Managing Emerging Contaminants in Drinking Water and Wastewater – A European Perspective, Emerging Contaminant Summit, Denver, Colorado (USA), 1st March 2016

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Letzel, T., Bieber, S., SFC-API-MS: Polarity Extended Separation Technique in Suspects Screening, SFC 2016, Vienna (AT), 6th – 7th October 2016

2017

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Bieber, S., Letzel, T., SFC und RPLC-HILIC mit massenspektrometrischer Detektion - Polaritätserweitertes Spurenstoffscreening in Gewässerproben, APPLICA 2017, Basel (CH), 7th September 2017

Poster:

2014

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2015

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2016

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2017

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Appendix

Supporting Information to Chapter 4

Management strategies for trace organic chemicals in water – a review of international approaches

Appendix

Table S- 1: Sources for water quality standards for organic chemicals, applied in the United States

Protected endpoint / waterbody	Source
Drinking and groundwater	https://www.epa.gov/ground-water-and-drinking-water/table-regulated-drinking-water-contaminants
Aquatic life	https://www.epa.gov/wqc/national-recommended-water-quality-criteria-aquatic-life-criteria-table
Human health (ambient water)	https://www.epa.gov/wqc/national-recommended-water-quality-criteria-human-health-criteria-table

Table S- 2: Environmental quality standards (EQS) for organic chemicals, applied in the European Union (European Commission, 2013). Priority hazardous substances are marked in bold. Inorganic chemicals, considered to pose a risk to the environment in the EU are excluded.

Compound name	**CAS number (1)**	**AA-EQS (2) (freshwater) [µg/L]**	**MAC-EQS (3) (freshwater) [µg/L]**	**EQS (biota) [µg/kg wet weight]**
Alachlor	15972-60-8	0.3	0.7	
Anthracene	120-12-7	0.1	0.1	
Atrazine	1912-24-9	0.6	2.0	
Benzene	71-43-2	10	50	
Brominated diphenylethers (4)	32534-81-9		0.14	0.0085
Carbon-tetrachloride (6)	56-23-5	12	not applicable	
C10-13 Chloroalkanes (7)	85535-84-8	0.4	1.4	
Chlorfenvinphos	470-90-6	0.1	0.3	
Chlorpyrifos (Chlorpyrifosethyl)	2921-88-2	0.03	0.1	
Cyclodiene pesticides: (6) Aldrin, Dieldrin, Endrin, Isodrin	309-00-2 60-57-1 72-20-8 465-73-6	$\Sigma = 0.01$	not applicable	
DDT total (6,8) para-para- DDT (6)	not applicable 50-29-3	0.025 0.01	not applicable not applicable	
1,2-Dichloroethane	107-06-2	10	not applicable	
Dichloromethane	75-09-2	20	not applicable	
Di(2-ethylhexyl)-phthalate (DEHP)	117-81-7	1.3	not applicable	
Diuron	330-54-1	0.2	1.8	
Endosulfan	115-29-7	0.005	0.01	
Fluoranthene	206-44-0	0.0063	0.12	30
Hexachlorobenzene	118-74-1		0.05	10
Hexachlorobutadiene	87-68-3		0.6	55
Hexachlorocyclohexane	608-73-1	0.02	0.04	
Isoproturon	34123-59-6	0.3	1.0	
Naphthalene	91-20-3	2	130	
Nonylphenols (4-Nonylphenol)	84852-15-3	0.3	2.0	
Octylphenols ((4-(1,1‘ ,3,3‘-tetramethylbutyl)-phenol))	140-66-9	0.1	not applicable	
Pentachlorobenzene	608-93-5	0.007	not applicable	
Pentachlorophenol	87-86-5	0.4	1	

Polyaromatic hydrocarbons (PAH) (11)				
Benzo(a)pyrene	50-32-8	1.7×10^{-4}	0.27	5
Benzo(b)fluoranthene	205-99-2	Footnote 10	0.017	Footnote 10
Benzo(k)fluoranthene	207-08-9	Footnote 10	0.017	Footnote 10
Benzo(g,h,i)perylene	191-24-2	Footnote 10	8.2×10^{-3}	Footnote 10
Indeno(1,2,3- cd)-pyrene	193-39-5	Footnote 10	not applicable	Footnote 10
Simazine	122-34-9	1	4	
Tetrachloroethylene (6)	127-18-4	10	not applicable	
Trichloroethylene (6)	79-01-6	10	not applicable	
Tributyltin compounds (Tributyltin-cation)	36643-28-4	0.0002	0.0015	
Trichlorobenzenes	12002-48-1	0.4	not applicable	
Trichloromethane	67-66-3	2.5	not applicable	
Trifluralin	1582-09-8	0.03	not applicable	
Dicofol	115-32-2	1.3×10^{-3}	not applicable (9)	33
Perfluorooctane sulfonic acid and its derivatives (PFOS)	1763-23-1	6.5×10^{-4}	36	9.1
Quinoxyfen	124495-18-7	0.15	2.7	
Dioxins and dioxin-like compounds	Footnote 11		not applicable (9)	Sum of PCDD+PCDF+ PCB-DL 0,0065 µg.kg –1 TEQ (12)
Aclonifen	74070-46-5	0.12	0.12	
Bifenox	42576-02-3	0.012	0.04	
Cybutryne	28159-98-0	0.0025	0.016	
Cypermethrin	52315-07-8	8×10^{-5}	6×10^{-4}	
Dichlorvos	62-73-7	6×10^{-4}	7×10^{-4}	
Hexabromocyclododecane (HBCDD)	Footnote 11	0.0016	0.5	167
Heptachlor and heptachlor epoxide	76-44- 8 1024-57-3	2×10^{-7}	3×10^{-4}	6.7×10^{-3}
Terbutryn	886-50-0	0.065	0.34	

Table S- 3: Compounds which have to be monitored in all waterbodies of the EU (watch list) (European Commission, 2015a)

Compound name	**CAS number** (1)	**Analytical detection limit [ng/L]**
17-Alpha-ethinylestradiol (EE2)	57-63-6	0.035
17-Beta-estradiol (E2), Estrone (E1)	50-28-2, 53-16-7	0.4
Diclofenac	15307-86-5	10
2,6-Ditert-butyl-4-methylphenol 2-Ethylhexyl	128-37-0	3160
2-Ethylhexyl 4-methoxycinnamate	5466-77-3	6000
Macrolide antibiotics		90
Erythromycin	114-07-8	
Clarithromycin	81103-11-9	
Azithromycin	83905-01-5	
Methiocarb	2032-65-7	10
Neonicotinoids		9
Imidacloprid	105827-78-9/138261-41-3	
Thiacloprid	111988-49-9	
Thiamethoxam	153719-23-4	
Clothianidin	210880-92-5	
Acetamiprid	135410-20-7/160430-64-8	
Oxadiazon	19666-30-9	88
Tri-allate	2303-17-5	670

Table S- 4: Indicator TOrC selected in Switzerland to assess efficiency of ozonation and PAC, divided in two categories due to removal efficiencies by ozonation or PAC (Götz et al., 2015)

Indicator compound with a removal efficiency for ozonation or adsorption of > 80%	Indicator compound with a removal efficiency for ozonation or adsorption of 60-80%
Amisulpride	Benzotriazole
Carbamazepine	Candesartane
Citalopram	Irbesartane
Clarithromycin	Mecoprop
Diclofenac	
Hydrochlorothiazide	
Metoprolol	

Table S- 5: Swiss specific trace organic compounds (high detection rate, occasionally detected high concentrations or specific toxicity) (BAFU, 2015)

Compound name	CAS number (1)		Compound name	CAS number (1)
2,4-D	94-75-7		Imidacloprid	138261-41-3
Acesulfam	55589-62-3		Iomeprol	78649-41-9
AMPA	1066-51-9		Iopamidol	62883-00-5
Atenolol	29122-68-7		Iopromid	73334-07-3
Atrazine	1912-24-9		Iprovalicarb	140923-17-7
Azithromycin	83905-01-5		Irgarol	28159-98-0
Azoxystrobin	131860-33-8		Isoproturon	34123-59-6
Bentazon	25057-89-0		Linuron	330-55-2
Benzothiazol	95-16-9		MCPA	94-74-6
Benzotriazol	95-14-7		Mecoprop-P	16484-77-8
Bezafibrat	41859-67-0		Mefenamic	61-68-7
Bisphenol	80-05-7		Metalaxyl-M	70630-17-0
Boscalid	188425-85-6		Metamitron	41394-05-2
Carbamazepin	298-46-4		Metazachlor	67129-08-2
Carbamazepin-10,11	58955-93-4		Metformin	657-24-9
Carbendazim	10605-21-7		Methoxyfenozid	161050-58-4
Chloridazon	1698-60-8		Methylbenzotriazol	136-85-6
Chloridazon-desphenyl	6339-19-1		Metoprolol	37350-58-6
Chlorotoluron	15545-48-9		Metribuzin	21087-64-9
Chlorpyrifos	2921-88-2		N4-Acetylsulfamethoxazol	21312-10-7
Chlorpyrifos-methyl	5598-13-0		Napropamide	15299-99-7
Clarithromycin	81103-11-9		Naproxen	22204-53-1
Cypermethrin	52315-07-8		Nicosulfuron	111991-09-4
Cyproconazole	94361-06-5		Nonylphenol	104-40-5
Cyprodinil	121552-61-2		NTA	139-13-9
DEET	134-62-3		Perfluoroctansulfonat	1763-23-1

Diatrizoat	117-96-4		Pirimicarb	23103-98-2
Diazinon	333-41-5		Propamocarb	24579-73-5
Dicamba	1918-00-9		Pyrimethanil	53112-28-0
Diclofenac	15307-86-5		S-Metolachlor	87392-12-9/178961-20-1
Dimethoate	60-51-5		Sotalol	3930-20-9
Diuron	330-54-1		Sucralose	56038-13-2
EDTA	60-00-4		Sulfamethazin	57-68-1
Epoxiconazole	133855-98-8/106325-08-0		Sulfamethoxazole	723-46-6
Erythromycin	114-07-8		Tebuconazole	107534-96-3
Estradiol	50-28-2		Terbuthylazine	5915-41-3
Estron	53-16-7		Terbutryn	886-50-0
Ethinylestratdiol	57-63-6		Thiacloprid	111988-49-9
Ethofumesate	26225-79-6		Thiamethoxam	153719-23-4
Glyphosat	1071-83-6		Triclosan	3380-34-5
Ibuprofen	15687-27-1		Trimethoprim	738-70-5

Table S- 6: Guideline values for organic contaminants of recycled water, which require consideration in environmental risk assessment. Only compounds with assigned concentration values are listed. The full list of compounds can be obtained from (NWQMS Phase 1, 2006)**.**

Compound name	**Threshold level for a protection of 99% of species [µg/L]**		**Compound name**	**Threshold level for a protection of 99% of species [µg/L]**
1,1,2-trichloroethane	5,400		Diazinon	0.00003
1,2,3-trichlorobenzene	3		Dibutylphthalate	9.9
1,2,4-trichlorobenzene	85		Diethylphthalate	900
1,2-dichlorobenzene	120		Dimethoate	0.1
1,3-dichlorobenzene	160		Dimethylphthalate	3000
1,4-dichlorobenzene	40		Diquat	0.01
2,3,4,6- tetrachlorophenol	10		Endosulfan	0.03
2,4,5-T	3		Endrin	0.01
2,4,6-trichlorophenol	3		Ethanol	400
2,4,6-trinitrotoluene	100		Fenitrothion	0.1
2,4-D	140		Glyphosate	370
2,4-dichloroaniline	0.6		Heptachlor	0.01
2,4-dichloropheno	120		Hexachloroethane	290
2,4-dinitrophenol	13		Lindane	0.07
2,4-dinitrotoluene	16		Linear alkylbenzene sulfonates (LAS)	65
2-chlorophenol	340		Malathion	0.0007
3,4-dichloroaniline	1.3		Methomyl	0.5
4-chloropheno	160		Molinate	0.1
Alcohol ethoxylated surfactants (AE)	50		Naphthalene	2.5
Alcohol ethoxyolated sulfate (AES)	340		Nitrobenzene	230
Aniline	8		o-xylene	200
Aroclor 1242	0.3		Pentachlorophenol	3.6
Aroclor 1254	0.01		Phenol	85
Atrazine	0.7		Poly(acrylonitrile-co-butadiene-costyrene)	200

Azinphos methyl	0.01		p-xylene	140
Benzene	600		Simazine	0.2
Carbofuran	0.06		Tebuthiuron	0.02
Chlordane	0.03		Thiobencarb	1
Chlorpyrifos	0.00004		Thiram	0.01
DDT	0.006		Toxaphene	0.1
			Trifluralin	2.6

Footnotes:

(1) CAS: Chemical Abstracts Service.

(2) This parameter is the EQS expressed as an annual average value (AA-EQS). Unless otherwise specified, it applies to the total concentration of all isomers.

(3) This parameter is the EQS expressed as a maximum allowable concentration (MAC-EQS). Where the MAC-EQS are marked as “not applicable”, the AA-EQS values are considered protective against short-term pollution peaks in continuous discharges since they are significantly lower than the values derived on the basis of acute toxicity.

(4) For the group of priority substances covered by brominated diphenylethers, the EQS refers to the sum of the concentrations of congener numbers 28, 47, 99, 100, 153 and 154.

(6) This substance is not a priority substance but one of the other pollutants for which the EQS are identical to those laid down in the legislation that applied prior to 13 January 2009.

(7) No indicative parameter is provided for this group of substances. The indicative parameter(s) must be defined through the analytical method.

(8) DDT total comprises the sum of the isomers 1,1,1-trichloro-2,2 bis (p-chlorophenyl) ethane (CAS number 50-29-3; EU number 200-024-3); 1,1,1-trichloro-2(o-chlorophenyl)-2-(p-chlorophenyl) ethane (CAS number 789-02-6; EU Number 212-332-5); 1,1-dichloro-2,2 bis (p-chlorophenyl) ethylene (CAS number 72-55-9; EU Number 200-784-6); and 1,1-dichloro-2,2 bis (p-chlorophenyl) ethane (CAS number 72-54-8; EU Number 200-783-0).

(9) There is insufficient information available to set a MAC-EQS for these substances.

(10) For the group of priority substances of polyaromatic hydrocarbons (PAH) (No 28), the biota EQS and corresponding AA-EQS in water refer to the concentration of benzo(a)pyrene, on the toxicity of which they are based. Benzo(a)pyrene can be considered as a marker for the other PAHs, hence only benzo(a)pyrene needs to be monitored for comparison with the biota EQS or the corresponding AA- EQS in water.

(11) Unless otherwise indicated, the biota EQS relate to fish. An alternative biota taxon, or another matrix, may be monitored instead, as long as the EQS applied provides an equivalent level of protection. For substances numbered 15 (Fluoranthene) and 28 (PAHs), the biota EQS refers to crustaceans and molluscs. For the purpose of assessing chemical status, monitoring of Fluoranthene and PAHs in fish is not appropriate. For substance number 37 (Dioxins and dioxin-like compounds), the biota EQS relates to fish, crustaceans and molluscs, in line with section 5.3 of the Annex to Commission Regulation (EU) No 1259/2011 of 2 December 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for dioxins, dioxin-like PCBs and non-dioxin-like PCBs in foodstuffs:

(12) PCDD: polychlorinated dibenzo-p-dioxins; PCDF: polychlorinated dibenzofurans; PCB-DL: dioxin-like polychlorinated biphenyls; TEQ: toxic equivalents according to the World Health Organisation 2005 Toxic Equivalence Factors.

Supporting Information to Chapter 6

RPLC-HILIC and SFC with mass spectrometry: Polarity-extended organic molecule screening in environmental (water) samples

Table S- 7: Standard compounds (including Empirical Formula, Monoisotopic Mass, logD (pH 7) and (pH 5), CAS Number, Compound Source and Distributor) utilized in the present study for the separation with RPLC-HILIC and SFC. Compounds are grouped in very polar (i.e. logD (pH 7) -7.71 to -2.5), polar (i.e. logD -2.5 to 2) and non-polar (i.e. logD 2 to 7.76) compounds and sorted by log D within groups. Compound source categories were biocides, pesticides, pharmaceuticals, personal care products (PCP) pesticides, flame retardants, transformation products (TP), REACH chemicals and natural organic matter (NOM).

	Compound name	IUPAC name	CAS Number	Empirical Formula	Monoisotopic Mass	log D (pH 5)	log D (pH 7)	Source	Distributor
Very polar	N,N-Bis(3-aminopropyl)methylamine	bis(3-aminopropyl)(methyl)amine	105-83-9	C7H19N3	145.1579	-10.06	-7.71	REACH-chemical	Sigma-Aldrich
	Glyphosate	2-[(phosphonomethyl)amino]acetic acid	1071-83-6	C3H8NO5P	169.0140	-5.12	-6.9	TP	Sigma-Aldrich
	1,3,4,5-Tetrahydroxycyclohexanecarboxylic acid	1,3,4,5-tetrahydroxycyclohexane-1-carboxylic acid	77-95-2	C7H12O6	192.0634	-4.24	-5.93	Biocides	Aldrich
	Cystine	(2R)-2-amino-3-{[(2R)-2-amino-2-carboxyethyl]disulfanyl}propanoic acid	56-89-3	C6H12N2O4S2	240.0238	-5.9	-5.9	REACH-chemical	Sigma-Aldrich
	Glutamic acid	2-aminopentanedioic acid	617-65-2	C5H9NO4	147.0532	-4	-5.86	NOM	Sigma-Aldrich
	Moroxydine	N-(diaminomethylidene)morpholine-4-carboximidamide	3731-59-7	C6H13N5O	171.1120	-6.01	-5.43	Uncategorized	Sigma-Aldrich
	Aspartic acid	2-aminobutanedioic acid	617-45-8	C4H7NO4	133.0375	-3.73	-5.37	REACH-chemical	Fluka
	Clavulanate	(2R,3Z,5R)-3-(2-hydroxyethylidene)-7-oxo-4-oxa-1-azabicyclo[3.2.0]heptane-2-carboxylic acid	58001-44-8	C8H9NO5	199.0481	-3.21	-4.82	Pharmaceuticals / TP	Sigma-Aldrich
	Oxaceprol	(2S,4S)-1-acetyl-4-hydroxypyrrolidine-2-carboxylate	33996-33-7	C7H11NO4	173.0688	-2.96	-4.74	Pharmaceuticals	Sigma-Aldrich
	Maleic acid	(2Z)-but-2-enedioic acid	110-16-7	C4H4O4	116.0110	-2.04	-4.55	REACH-chemical	Merck
	Tetracycline	2-(Amino-hydroxy-methylidene)-4-dimethylamino-6,10,11,12a-tetrahydroxy-6-methyl-4,4a,5,5a-tetrahydrotetracene-1,3,12-trione	60-54-8	C22H24N2O8	444.1533	-2.65	-4.33	TP	Sigma-Aldrich
	L-Asparagine	2-amino-3-carbamoylpropanoic acid	70-47-3	C4H8N2O3	132.0535	-4.29	-4.3	NOM	Fluka
	Cefuroxime	(6R,7R)-3-[(carbamoyloxy)methyl]-7-[(2E)-2-(furan-2-yl)-2-(methoxyimino)acetamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid	55268-75-2	C16H16N4O8S	424.0689	-2.75	-4.26	TP	Sigma-Aldrich
	Acetylcholine	2-acetyloxyethyl(trimethyl)azanium	51-84-3	C7H16NO2	146.1176	-4.22	-4.22	REACH-chemical	Acros Organics
	Acamprosate	3-acetamidopropane-1-sulfonate	77337-76-9	C5H11NO4S	181.0409	-4.1	-4.1	Pharmaceutical	Sigma-Aldrich
	Aminomethyl propanediol	2-amino-2-methylpropane-1,3-diol	115-69-5	C4H11NO2	105.0790	-4.68	-4.04	Uncategorized	Sigma-Aldrich

	Miglitol	(2R,3R,4R,5S)-1-(2-hydroxyethyl)-2-(hydroxymethyl)piperidine-3,4,5-triol	72432-03-2	C8H17NO5	207.1107	-5.74	-3.89	Uncategorized	Sigma-Aldrich
	Diethanolamine	2-[(2-hydroxyethyl)amino]ethan-1-ol	111-42-2	C4H11NO2	105.0790	-4.78	-3.79	Pharmaceuticals/ TP	Sigma-Aldrich
	Phenformin	1-(diaminomethylidene)-2-(2-phenylethyl)guanidine	114-86-3	C10H15N5	205.1327	-4.1	-3.76	Uncategorized	Sigma-Aldrich
	Betaine	2-(trimethylazaniumyl)acetate	107-43-7	C5H11NO2	117.0790	-3.72	-3.72	NOM	Sigma-Aldrich
	2-(2-Aminoethoxy)ethanol	2-(2-aminoethoxy)ethan-1-ol	929-06-6	C4H11NO2	105.0790	-4.38	-3.71	Pharmaceuticals	Sigma-Aldrich
	Histidine	2-amino-3-(1H-imidazol-5-yl)propanoic acid	4998-57-6	C6H9N3O2	155.0695	-4.17	-3.7	Uncategorized	Merck
	Metformin	2-(N,N-Dimethylcarbamimidoyl)guanidine	657-24-9	C4H11N5	129.1014	-3.66	-3.66	Pharmaceutical	Fluka
	1H-Imidazole-1-propylamine	3-(1H-imidazol-1-yl)propan-1-amine	5036-48-6	C6H11N3	125.0953	-4.26	-3.65	REACH-chemical	Sigma-Aldrich
	1-(3'-Carboxypropyl)-3-7-dimethylxanthin	4-(3,7-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-1-yl)butanoic acid	6493-07-8	C11H14N4O4	266.1015	-1.8	-3.6	TP	Sigma-Aldrich
	Tenofovir	[(2R)-1-(6-aminopurin-9-yl)propan-2-yl]oxymethylphosphonic acid	147127-20-6	C9H14N5O4P	287.0783	-3.71	-3.49	Uncategorized	Sigma-Aldrich
	Threonine	2-amino-3-hydroxybutanoic acid	80-68-2	C4H9NO3	119.0582	-3.47	-3.47	Uncategorized	Sigma-Aldrich
	Aminomethylpropanol	2-amino-2-methylpropan-1-ol	124-68-5	C4H11NO	89.0841	-3.65	-3.35	Pharmaceuticals	Sigma-Aldrich
	Fructose	(3S,4R,5R)-1,3,4,5,6-pentahydroxyhexan-2-one	57-48-7	C6H12O6	180.0634	-3.27	-3.27	NOM	Sigma-Aldrich
	Methylscopolamine	(1S,2R,4S,5S)-7-[(3-hydroxy-2-phenylpropanoyl)oxy]-9,9-dimethyl-3-oxa-9-9-azatricyclo[3.3.1.0^{2,4}]nonan-9-ium	155-41-9	C18H24NO4	318.1700	-3.27	-3.27	TP	Sigma-Aldrich
	2-Ethylaminoethanol	2-(ethylamino)ethan-1-ol	110-73-6	C4H11NO	89.0841	-3.76	-3.26	REACH-chemica	Sigma-Aldrich
	1,1'-Iminodipropan-2-Ol	1-[(2-hydroxypropyl)amino]propan-2-ol	110-97-4	C6H15NO2	133.1103	-3.96	-3.21	Pharmaceuticals	Sigma-Aldrich
	Sarcosine	2-(methylamino)acetic acid	107-97-1	C3H7NO2	89.0477	-3.19	-3.19	Pharmaceuticals	Sigma-Aldrich
	Glycine	2-aminoacetic acid	56-40-6	C2H5NO2	75.0320	-3.41	-3.18	Pharmaceuticals	Merck
	1-(N,N-Bis(2-hydroxyethyl)amino)propan-2-ol	1-[bis(2-hydroxyethyl)amino]propan-2-ol	6712-98-7	C7H17NO3	163.1208	-4.76	-3.18	REACH-chemical	Sigma-Aldrich
	beta-Alanine	3-aminopropanoic acid	107-95-9	C3H7NO2	89.0477	-3.21	-3.17	Pharmaceuticals	Sigma-Aldrich
	Acetyllysine	2-amino-6-acetamidohexanoic acid	692-04-6	C8H16N2O3	188.1161	-3.15	-3.15	NOM	Sigma-Aldrich

	Lisinopril	(2S)-1-[(2S)-6-amino-2-{[(1S)-1-carboxy-3-phenylpropyl]amino}hexanoyl]pyrrolidine-2-carboxylic acid dihydrate	76547-98-3	C21H31N3O5	405.2264	-3.12	-3.13	Uncategorized	Sigma-Aldrich
	Chlortetracycline	(4S,4aS,5aS,6S,12aS)-7-chloro-4-(dimethylamino)-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide	57-62-5	C22H23ClN2O8	478.1143	-2.89	-3.09	Pharmaceuticals / TP	Sigma-Aldrich
	Acesulfame	6-methyl-2,4-dioxo-4H-1,2$l^{6},3-oxathiazin-2-olate	33665-90-6	C4H5NO4S	162.9939	-2.97	-3.06	Uncategorized	Sigma-Aldrich
	3-Ethoxypropylamine	3-ethoxypropan-1-amine	6291-85-6	C5H13NO	103.0997	-3.29	-3.04	REACH-chemical	Sigma-Aldrich
	Famotidine	2-[4-[2-(Amino-sulfamoylimino-methyl)ethylsulfanylmethyl]-1,3-thiazol-2-yl]guanidine	76824-35-6	C8H15N7O2S3	337.0449	-3.43	-3.04	Pharmaceuticals / TP	Sigma-Aldrich
	Trans-4-aminocyclohexan-1-ol	4-aminocyclohexan-1-ol	27489-62-9	C6H13NO	115.0997	-3.24	-3	Pharmaceuticals	Sigma-Aldrich
	Glucose	6-(hydroxymethyl)oxane-2,3,4,5-tetrol	50-99-7	C6H12O6	180.0634	-2.93	-2.93	Uncategorized	Merck
	Gamma-aminobutyric acid	4-aminobutanoic acid	56-12-2	C4H9NO2	103.0633	-2.99	-2.89	Uncategorized	Sigma-Aldrich
	Triisopropanolamine	1-[bis(2-hydroxypropyl)amino]propan-2-ol	122-20-3	C9H21NO3	191.1521	-4.06	-2.88	Pharmaceuticals	Sigma-Aldrich
	L-Alanine	2-aminopropanoic acid	56-41-7	C3H7NO2	89.0477	-2.84	-2.84	NOM	Sigma-Aldrich
	2-Pyrrolidin-1-ylethanol	2-(pyrrolidin-1-yl)ethan-1-ol	2955-88-6	C6H13NO	115.0997	-3.57	-2.69	REACH-chemical	Sigma-Aldrich
	Dexrazoxane	4-[2-(3,5-dioxopiperazin-1-yl)propyl]piperazine-2,6-dione	21416-67-1	C11H16N4O4	268.1172	-2.67	-2.65	Pharmaceuticals	Sigma-Aldrich
	Taurine	2-aminoethane-1-sulfonic acid	107-35-7	C2H7NO3S	125.0147	-2.61	-2.62	PCP	Fluka
	Diethylamine	diethylamine	109-89-7	C4H11N	73.0891	-2.72	-2.56	Pharmaceuticals/ TP	Sigma-Aldrich
	Ectoine	2-methyl-3,4,5,6-tetrahydropyrimidine-4-carboxylic acid	96702-03-3	C6H10N2O2	142.0742	-2.53	-2.53	NOM	Sigma-Aldrich
Polar	Sotalol	N-[4-(1-Hydroxy-2-propan-2-ylamino-ethyl)phenyl]methanesulfonamide	3930-20-9	C12H20N2O3S	272.1195	-3.18	-2.47	Pharmaceutical	Sigma-Aldrich
	Amoxicillin	6-[[2-Amino-2-(4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid	26787-78-0	C16H19N3O5S	365.1045	-2.31	-2.43	Pharmaceutical	Sigma-Aldrich
	2-[2-(Dimethylamino)ethoxy]ethanol	2-[2-(dimethylamino)ethoxy]ethan-1-ol	1704-62-7	C6H15NO2	133.1103	-3.89	-2.41	REACH-chemical	Sigma-Aldrich
	2-Butylaminoethanol	2-(butylamino)ethan-1-ol	111-75-1	C6H15NO	117.1154	-2.79	-2.37	REACH-chemical	Sigma-Aldrich
	Allantoin	(2,5-dioxoimidazolidin-4-yl)urea	97-59-6	C4H6N4O3	158.0440	-2.36	-2.37	PCP	Sigma-Aldrich

	Piperidine	piperidine	110-89-4	C5H11N	85.0891	-2.58	-2.36	REACH-chemical	Sigma-Aldrich
	Guanylurea	N-guanylurea	207300-86-5	C2H6N4O	102.0542	-3.82	-2.36		Sigma-Aldrich
	L-Proline	pyrrolidine-2-carboxylic acid	147-85-3	C5H9NO2	115.0633	-2.34	-2.34	NOM	Fluka
	2-(Dimethylamino)-2-methylpropan-1-ol	2-(dimethylamino)-2-methylpropan-1-ol	7005-47-2	C6H15NO	117.1154	-3.27	-2.34	REACH-chemical	Sigma-Aldrich
	2,2,6,6-Tetramethylpiperidin-4-ol	2,2,6,6-tetramethylpiperidin-4-ol	2403-88-5	C9H19NO	157.1467	-2.72	-2.34	Pharmaceuticals	Sigma-Aldrich
	D(-) Ribose	(3R,4R,5R)-oxane-2,3,4,5-tetrol	10257-32-6	C5H10O5	150.0528	-2.3	-2.3	NOM	Sigma-Aldrich
	Cefalexin	(6R,7R)-7-{[(2R)-2-amino-2-phenylacetyl]amino}- 3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene- 2-carboxylic acid	15686-71-2	C16H17N3O4S	347.0940	-2.15	-2.26	Pharmaceuticals TP	Sigma-Aldrich
	Minoxidil	6-amino-2-imino-4-(piperidin-1-yl)-1,2-dihydropyrimidin-1-ol	38304-91-5	C9H15N5O	209.1277	-2.68	-2.25	Pharmaceuticals / TP	Sigma-Aldrich
	L-Methionine	2-amino-4-methylsulfanylbutanoic acid	59-51-8	C5H11NO2S	149.0510	-2.19	-2.19	Uncategorized	Sigma-Aldrich
	Atenolol	2-[4-(2-Hydroxy-3-propan-2-ylamino-propoxy)phenyl]ethanamide	29122-68-7	C14H22N2O3	266.1630	-2.8	-2.14	Pharmaceutical	Sigma-Aldrich
	Butylamine	butan-1-amine	109-73-9	C4H11N	73.0891	-2.33	-2.11	Pharmaceuticals	Sigma-Aldrich
	1,2,2,6,6-Pentamethylpiperidin-4-ol	1,2,2,6,6-pentamethylpiperidin-4-ol	2403-89-6	C10H21NO	171.1623	-2.59	-2.11	Pharmaceuticals	Sigma-Aldrich
	Adenosine	2-(6-amino-9H-purin-9-yl)-5-(hydroxymethyl)oxolane-3,4-diol	58-61-7	C10H13N5O4	267.0968	-2.38	-2.1	NOM	Merck
	Vigabatrin	4-aminohex-5-enoic acid	60643-86-9	C6H11NO2	129.0790	-2.21	-2.09	TP	Sigma-Aldrich
	Melamine	1,3,5-Triazine-2,4,6-triamine	108-78-1	C3H6N6	126.0654	-2.54	-2.02	REACH-chemical	Aldrich
	6-Amino-5-formamido-1-3-dimethyluracil	N-(6-amino-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)formamide	7597-60-6	C7H10N4O3	198.0753	-2.02	-2.02	REACH-chemical	Sigma-Aldrich
	Valine	2-amino-3-methylbutanoic acid	516-06-3	C5H11NO2	117.0790	-1.95	-1.95	NOM	Sigma-Aldrich
	Butylscopolamine	(1S,2R,4S,5S)-9-butyl-7-[(3-hydroxy-2-phenylpropanoyl)oxy]-9-methyl-3-oxa-9-azatricyclo[3.3.1.$0^{2,4}$]nonan-9-ium	149-64-4	C21H30NO4	360.2169	-1.94	-1.94	TP	Sigma-Aldrich
	Syringic acid	4-hydroxy-3,5-dimethoxybenzoic acid	530-57-4	C9H10O5	198.0528	-0.09	-1.93	Uncategorized	Sigma-Aldrich
	Octopamine	4-[(1R)-2-amino-1-hydroxyethyl]phenol	104-14-3	C8H11NO2	153.0790	-2.83	-1.92	TP	Sigma-Aldrich
	N,N-Dimethylisopropylamine	dimethyl(propan-2-yl)amine	996-35-0	C5H13N	87.1048	-2.52	-1.9	REACH-chemical	Sigma-Aldrich

	Oxypurinol	1H,2H,4H,5H,6H-pyrazolo[3,4-d]pyrimidine-4,6-dione	2465-59-0	C5H4N4O2	152.0334	-1.67	-1.88	TP	Sigma-Aldrich
	Norfenefrine	3-(2-amino-1-hydroxyethyl)phenol	536-21-0	C8H11NO2	153.0790	-2.83	-1.88	TP	Sigma-Aldrich
	N-[3-(Dimethylamino)propyl]methacrylamide	N-[3-(dimethylamino)propyl]-2-methylprop-2-enamide	5205-93-6	C9H18N2O	170.1419	-3.01	-1.85	Pharmaceuticals	Sigma-Aldrich
	Isopentylamine	3-methylbutan-1-amine	107-85-7	C5H13N	87.1048	-2.04	-1.82	REACH-chemical	Sigma-Aldrich
	N,N'-Ethylenedi(diacetamide)	N-(2-acetamidoethyl)acetamide	871-78-3	C6H12N2O2	144.0899	-1.78	-1.78	Pharmaceuticals	Sigma-Aldrich
	Diisopropylamine	N-(propan-2-yl)propan-2-amine	108-18-9	C6H15N	101.1204	-1.89	-1.76	Pharmaceuticals/ TP	Sigma-Aldrich
	Dopamine	4-(2-aminoethyl)benzene-1,2-diol	51-61-6	C8H11NO2	153.0790	-2.24	-1.75	Pharmaceuticals	Sigma-Aldrich
	Panthenol	2,4-dihydroxy-N-(3-hydroxypropyl)-3,3-dimethylbutanamide	16485-10-2	C9H19NO4	205.1314	-1.7	-1.7	REACH-chemical	Sigma-Aldrich
	Cyanurodiamide (Atrazin-desethyl-desisopropyl-2-hydroxy)	4,6-diamino-2,5-dihydro-1,3,5-triazin-2-one	645-92-1	C3H5N5O	127.0494	-1.69	-1.69	TP	Sigma-Aldrich
	Dacarbazine	(4E)-4-(3,3-dimethyltriazan-1-ylidene)-4H-imidazole-5-carboxamide	4342-03-4	C6H10N6O	182.0916	-1.69	-1.69	Pharmaceutical	Sigma-Aldrich
	Piracetam	2-(2-oxopyrrolidin-1-yl)acetamide	7491-74-9	C6H10N2O2	142.0742	-1.68	-1.68	Pharmaceutical	Sigma-Aldrich
	Ethylenediaminetetraacetonitrile	2-({2-[bis(cyanomethyl)amino]ethyl}(cyanomethyl)amino)acetonitrile	5766-67-6	C10H12N6	216.1123	-1.65	-1.65	REACH-chemical	Sigma-Aldrich
	Dipropylamine	dipropylamine	142-84-7	C6H15N	101.1204	-1.67	-1.56	Pharmaceuticals	Sigma-Aldrich
	Tranexamic acid	4-(aminomethyl)cyclohexane-1-carboxylic acid	1197-18-8	C8H15NO2	157.1103	-1.66	-1.55	Uncategorized	Sigma-Aldrich
	4-Hydroxybenzenesulfonic acid	4-hydroxybenzene-1-sulfonic acid	98-67-9	C6H6O4S	173.9987	-1.53	-1.53	Pharmaceuticals	Sigma-Aldrich
	Tyramine	4-(2-aminoethyl)phenol	51-67-2	C8H11NO	137.0841	-1.94	-1.52	Uncategorized	Sigma-Aldrich
	L-Isoleucine	(2S,3S)-2-amino-3-methylpentanoic acid	73-32-5	C6H13NO2	131.0946	-1.51	-1.51	NOM	Sigma-Aldrich
	Acetamide MEA	N-(2-hydroxyethyl)acetamide	142-26-7	C4H9NO2	103.0633	-1.5	-1.5	Uncategorized	Sigma-Aldrich
	L-Tyrosine	(2S)-2-amino-3-(4-hydroxyphenyl)propanoic acid	60-18-4	C9H11NO3	181.0739	-1.49	-1.49	NOM	Fluka
	Etilefrine	3-[2-(ethylamino)-1-hydroxyethyl]phenol	709-55-7	C10H15NO2	181.1103	-2.26	-1.42	Pharmaceutical	Sigma-Aldrich
	Dimethylsulfoxide	methanesulfinylmethane	67-68-5	C2H6OS	78.0139	-1.41	-1.41	Pharmaceuticals/ TP	Sigma-Aldrich

	N-(Hydroxymethyl)Acetamide	N-(hydroxymethyl)acetamide	625-51-4	C3H7NO2	89.0477	-1.39	-1.39	Biocides	Sigma-Aldrich
	Phenylpyruvic acid	2-oxo-3-phenylpropanoic acid	156-06-9	C9H8O3	164.0473	0.23	-1.39	Uncategorized	Sigma-Aldrich
	Hydroxycarbamide	hydroxyurea	127-07-1	CH4N2O2	76.0273	-1.37	-1.37	Pharmaceuticals/ TP	Sigma-Aldrich
	(2,4-Diaminopteridin-6-yl)yethanol	(2,4-diaminopteridin-6-yl)methanol	73978-41-3	C7H8N6O	192.0760	-1.37	-1.37	REACH-chemical	Sigma-Aldrich
	Urea	urea	57-13-6	CH4N2O	60.0324	-1.36	-1.36	Uncategorized	Merck
	Pregabalin	(3S)-3-(aminomethyl)-5-methylhexanoic acid	148553-50-8	C8H17NO2	159.1259	-1.51	-1.35	Pharmaceutical	Sigma-Aldrich
	Tetraethylene glycol	2-{2-[2-(2-hydroxyethoxy)ethoxy]ethoxy}ethan-1-ol	112-60-7	C8H18O5	194.1154	-1.35	-1.35	REACH-chemical	Sigma-Aldrich
	2-6-Dimethylmorpholine	2,6-dimethylmorpholine	141-91-3	C6H13NO	115.0997	-2.7	-1.33	Pharmaceuticals	Sigma-Aldrich
	Gabapentin	2-[1-(aminomethyl)cyclohexyl]acetic acid	60142-96-3	C9H17NO2	171.1259	-1.4	-1.27	Pharmaceutical	Sigma-Aldrich
	Ferulic acid	(E)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enoic acid	537-98-4	C10H10O4	194.0579	0.61	-1.24	Uncategorized	Sigma-Aldrich
	Atenolol acid (metoprolol acid)	2-(4-{2-hydroxy-3-[(propan-2-yl)amino]propoxy}phenyl)acetic acid	56392-14-4	C14H21NO4	267.1471	-1.25	-1.24	Pharmaceutical	Sigma-Aldrich
	Riboflavin	7,8-dimethyl-10-(2,3,4,5-tetrahydroxypentyl)-2H,3H,4H,10H-benzo[g]pteridine-2,4-dione	83-88-5	C17H20N4O6	376.1383	-0.92	-1.23	NOM	Merck
	Methacrylic Acid	2-methylprop-2-enoic acid	79-41-4	C4H6O2	86.0368	0.55	-1.21	Pharmaceuticals	Sigma-Aldrich
	3-(2-Aminopropyl)phenol	3-[(2S)-2-aminopropyl]phenol	18840-47-6	C9H13NO	151.0997	-1.53	-1.19	Pharmaceuticals	Sigma-Aldrich
	(R)-1-(4-methoxyphenyl)ethylamine	(1R)-1-(4-methoxyphenyl)ethanamine	22038-86-4	C9H13NO	151.0997	-1.67	-1.19	REACH-chemical	Sigma-Aldrich
	Phenylalanine	2-amino-3-phenylpropanoic acid	150-30-1	C9H11NO2	165.0790	-1.19	-1.19	NOM	Merck
	Cytosine	6-aminopyrimidin-2(1H)-one	71-30-7	C4H5N3O	111.0433	-1.15	-1.15	NOM	Sigma-Aldrich
	4-Methylmorpholine 4-oxide	4-methylmorpholin-4-ium-4-olate	7529-22-8	C5H11NO2	117.0790	-1.16	-1.15	REACH-chemical	Sigma-Aldrich
	Tolazoline	2-benzyl-4,5-dihydro-1H-imidazole	59-98-3	C10H12N2	160.1000	-1.21	-1.15	TP	Sigma-Aldrich
	Methylurea	methylurea	598-50-5	C2H6N2O	74.0480	-1.14	-1.14	REACH-chemical	Sigma-Aldrich
	Cyanuric acid	1,3,5-triazinane-2,4,6-trione	108-80-5	C3H3N3O3	129.0174	-1.04	-1.14	REACH-chemical	Fluka

	2-Morpholinoethanol	2-(morpholin-4-yl)ethan-1-ol	622-40-2	C6H13NO2	131.0946	-2.89	-1.13	REACH-chemical	Sigma-Aldrich
	Tryptophan	2-amino-3-(1H-indol-3-yl)propanoic acid	73-22-3	C11H12N2O2	204.0899	-1.09	-1.09	Uncategorized	Merck
	D-α-methylbenzylamine	(1R)-1-phenylethanamine	3886-69-9	C8H11N	121.0891	-1.51	-1.04	REACH-chemical	Sigma-Aldrich
	L-α-Methylbenzylamine	(1S)-1-phenylethan-1-amine	2627-86-3	C8H11N	121.0891	-1.51	-1.04	REACH-chemical	Sigma-Aldrich
	Cyanoguanidine	2-cyanoguanidine	461-58-5	C2H4N4	84.0436	-1.03	-1.03	Pharmaceuticals	Sigma-Aldrich
	Perhydropyrimidin-2-one	1,3-diazinan-2-one	1852-17-1	C4H8N2O	100.0637	-1.03	-1.03	Pharmaceuticals	Sigma-Aldrich
	Propylidynetrimethanol	2-ethyl-2-(hydroxymethyl)propane-1,3-diol	77-99-6	C6H14O3	134.0943	-1.01	-1.01	Pharmaceuticals	Sigma-Aldrich
	Ethyl glycinate	ethyl 2-aminoacetate	459-73-4	C4H9NO2	103.0633	-2.69	-0.99	REACH-chemical	Sigma-Aldrich
	Pyridoxine	4,5-bis(hydroxymethyl)-2-methylpyridin-3-ol	65-23-6	C8H11NO3	169.0739	-1.58	-0.97	TP	Sigma-Aldrich
	Emtricitabine	4-amino-5-fluoro-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one	143491-57-0	C8H10FN3O3S	247.0427	-0.9	-0.9	Pharmaceutical	Sigma-Aldrich
	P-Coumaric acid	(E)-3-(4-hydroxyphenyl)prop-2-enoic acid	7400-08-0	C9H8O3	164.0473	0.97	-0.89	Uncategorized	Sigma-Aldrich
	N-Formylmorpholine	Morpholine-4-carbaldehyde	4394-85-8	C5H9NO2	115.0633	-0.85	-0.85	TP	Sigma-Aldrich
	Vasotec	(2S)-1-[(2S)-2-{[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino}propanoyl]pyrrolidine-2-carboxylic acid	75847-73-3	C20H28N2O5	376.1998	0.55	-0.85	TP	Sigma-Aldrich
	2-Hydroxy-N,N-dimethyl-propanamide	2-hydroxy-N,N-dimethylpropanamide	35123-06-9	C5H11NO2	117.0790	-0.83	-0.83	REACH-chemical	Sigma-Aldrich
	N-(Hydroxymethyl)nicotinamide	N-(hydroxymethyl)pyridine-3-carboxamide	3569-99-1	C7H8N2O2	152.0586	-0.77	-0.75	Uncategorized	Sigma-Aldrich
	N-(2-Hydroxyethyl)prop-2-enamide	N-(2-hydroxyethyl)prop-2-enamide	7646-67-5	C5H9NO2	115.0633	-0.74	-0.74	REACH-chemica	Sigma-Aldrich
	1,4-Butinodiol	but-2-yne-1,4-diol	110-65-6	C4H6O2	86.0368	-0.73	-0.73	Pharmaceuticals	Sigma-Aldrich
	2-2'-Dimorpholinyldiethyl ether	4-{2-[2-(morpholin-4-yl)ethoxy]ethyl}morpholine	6425-39-4	C12H24N2O3	244.1787	-3.7	-0.71	REACH-chemical	Sigma-Aldrich
	Lenalidomide	3-(4-amino-1-oxo-2,3-dihydro-1H-isoindol-2-yl)piperidine-2,6-dione	191732-72-6	C13H13N3O3	259.0957	-0.71	-0.71	Pharmaceuticals	Cayman Chemical Company
	Isoniazid	pyridine-4-carbohydrazide	54-85-3	C6H7N3O	137.0589	-0.7	-0.69	Pharmaceutical	Sigma-Aldrich
	4-Methylmorpholine	4-methylmorpholine	109-02-4	C5H11NO	101.0841	-2.5	-0.66	REACH-chemical	Sigma-Aldrich

	Methoxytriglycol	2-[2-(2-methoxyethoxy)ethoxy]ethan-1-ol	112-35-6	C7H16O4	164.1049	-0.66	-0.66	Pharmaceuticals	Sigma-Aldrich
	1,3-Dimethylimidazolidin-2-one	1,3-dimethylimidazolidin-2-one	80-73-9	C5H10N2O	114.0793	-0.64	-0.64	REACH-chemical	Sigma-Aldrich
	Diatrizoate	3,5-Bis(acetylamino)-2,4,6-triiodo-benzoic acid	117-96-4	C11H9I3N2O4	613.7697	0.14	-0.62	TP	Sigma-Aldrich
	Arecoline	methyl 1-methyl-3,6-dihydro-2H-pyridine-5-carboxylate	63-75-2	C8H13NO2	155.0946	-2.39	-0.6	Pharmaceutical	Fluka
	2-Pyrrolidone	pyrrolidin-2-one	616-45-5	C4H7NO	85.0528	-0.58	-0.58	Pharmaceuticals	Sigma-Aldrich
	Adenine	7H-purin-6-amine	73-24-5	C5H5N5	135.0545	-1.05	-0.58	NOM	Sigma-Aldrich
	Asulam	methyl N-(4-aminobenzenesulfonyl)carbamate	3337-71-1	C8H10N2O4S	230.0361	-0.55	-0.58	TP	Sigma-Aldrich
	Hydrochlorthiazide	6-chloro-1,1-dioxo-3,4-dihydro-2H-1λ^6,2,4-benzothiadiazine-7-sulfonamide	58-93-5	C7H8ClN3O4S2	296.9645	-0.58	-0.58	Pharmaceuticals / TP	Sigma-Aldrich
	2-Methylimidazole	2-methyl-1H-imidazole	693-98-1	C4H6N2	82.0531	-1.08	-0.55	Pharmaceuticals	Sigma-Aldrich
	Omethoate	O,O-dimethyl S-[2-(methylamino)-2-oxoethyl] phosphorothioate	1113-02-6	C5H12NO4PS	213.0225	-0.55	-0.55	Pesticide	Fluka
	Sucralose	(2R,3R,4R,5R,6R)-2-{[(2R,3S,4S,5S)-2,5-bis(chloromethyl)-3,4-dihydroxyoxolan-2-yl]oxy}-5-chloro-6-(hydroxymethyl)oxane-3,4-diol	56038-13-2	C12H19Cl3O8	396.0146	-0.47	-0.47	TP	Sigma-Aldrich
	Iopromide	1-N,3-N-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-5-(2-methoxyacetamido)-1-N-methylbenzene-1,3-dicarboxamide	73334-07-3	C18H24I3N3O8	790.8698	-0.44	-0.44	Pharmaceutical	Sigma-Aldrich
	2-Ethylhexylamine	3-(aminomethyl)heptane	104-75-6	C8H19N	129.1517	-0.63	-0.42	Pharmaceuticals	Sigma-Aldrich
	Ethyl 2-oxopyrrolidine-1-acetate	ethyl 2-(2-oxopyrrolidin-1-yl)acetate	61516-73-2	C8H13NO3	171.0895	-0.38	-0.38	REACH-chemical	Sigma-Aldrich
	Ritalinic Acid	2-phenyl-2-(piperidin-2-yl)acetic acid	19395-41-6	C13H17NO2	219.1259	-0.38	-0.36	TP	Sigma-Aldrich
	Cimetidine	1-cyano-2-methyl-3-(2-{[(5-methyl-1H-imidazol-4-yl)methyl]sulfanyl}ethyl)guanidine	51481-61-9	C10H16N6S	252.1157	-1.24	-0.34	Pharmaceutical	Sigma-Aldrich
	2,2,6,6-Tetramethyl-4-piperidone	2,2,6,6-tetramethylpiperidin-4-one	826-36-8	C9H17NO	155.1310	-1.87	-0.32	Pharmaceuticals	Sigma-Aldrich
	Dorzolamide	(2S,4S)-4-(ethylamino)-2-methyl-1,1-dioxo-2H,3H,4H-1λ^6-thieno[2,3-b]thiopyran-6-sulfonamide	120279-96-1	C10H16N2O4S3	324.0272	-2.04	-0.32	Pharmaceutical	Sigma-Aldrich
	4-Methylimidazole	4-methyl-1H-imidazole	822-36-6	C4H6N2	82.0531	-0.53	-0.3	REACH-chemical	Sigma-Aldrich
	3-Dimethylaminopropiononitrile	3-(dimethylamino)propanenitrile	1738-25-6	C5H10N2	98.0844	-1.95	-0.3	Pharmaceuticals	Sigma-Aldrich

	Diethylene glycol ethyl ether	2-(2-ethoxyethoxy)ethan-1-ol	111-90-0	C6H14O3	134.0943	-0.26	-0.26	Pharmaceuticals	Sigma-Aldrich
	1,2-Dimethylimidazole	1,2-dimethyl-1H-imidazole	1739-84-0	C5H8N2	96.0687	-0.85	-0.25	REACH-chemical	Sigma-Aldrich
	Methyl 3-aminocrotonate	methyl (2E)-3-aminobut-2-enoate	14205-39-1	C5H9NO2	115.0633	-0.24	-0.24	REACH-chemical	Sigma-Aldrich
	6-Mercaptopurine	6,7-dihydro-3H-purine-6-thione	50-44-2	C5H4N4S	152.0157	-0.13	-0.12	Pharmaceuticals/ TP	Sigma-Aldrich
	Candesartan	2-ethoxy-1-({4-[2-(1H-1,2,3,4-tetrazol-5-yl)phenyl]phenyl}methyl)-1H-1,3-benzodiazole-7-carboxylic acid	139481-59-7	C24H20N6O3	440.1597	2.4	-0.12	Uncategorized	Alfa Aesar
	2-Mercaptoethanol	2-sulfanylethan-1-ol	60-24-2	C2H6OS	78.0139	-0.11	-0.11	Pharmaceuticals	Sigma-Aldrich
	[2-(Acryloyloxy)ethyl]trimethylammonium	2-(dimethylamino)ethyl 2-methylprop-2-enoate	2867-47-2	C8H15NO2	157.1103	-1.83	-0.1	Pharmaceuticals	Sigma-Aldrich
	1,4-Dioxane	1,4-dioxane	123-91-1	C4H8O2	88.0524	-0.09	-0.09	Pharmaceuticals	Sigma-Aldrich
	Hexanoic acid	hexanoic acid	142-62-1	C6H12O2	116.0837	1.55	-0.09	Biocides	Merck
	1-Methylimidazole	1-methyl-1H-imidazole	616-47-7	C4H6N2	82.0531	-0.49	-0.07	REACH-chemical	Sigma-Aldrich
	Tetraglyme	2,5,8,11,14-pentaoxapentadecane	143-24-8	C10H22O5	222.1467	-0.06	-0.06	REACH-chemical	Sigma-Aldrich
	Nicotinylalcohol	pyridin-3-ylmethanol	100-55-0	C6H7NO	109.0528	-0.17	-0.01	TP	Sigma-Aldrich
	2-Phenyl-1H-benzimidazole-5-sulphonic acid	2-phenyl-1H-1,3-benzodiazole-6-sulfonic acid	27503-81-7	C13H10N2O3S	274.0412	0	0.09	REACH-chemical	Sigma-Aldrich
	Pipamperone	1'-[4-(4-fluorophenyl)-4-oxobutyl]-1,4'-bipiperidine-4'-carboxamide	1893-33-0	C21H30FN3O2	375.2322	-2.57	0.13	Pharmaceutical	Sigma-Aldrich
	Sulfamethoxazole	4-amino-N-(5-methyl-1,2-oxazol-3-yl)benzene-1-sulfonamide	723-46-6	C10H11N3O3S	253.0521	0.76	0.14	Pharmaceutical	Fluka
	Naproxen	2-(6-Methoxynaphthalen-2-yl)propanoic acid	22204-53-1	C14H14O3	230.0943	2.11	0.25	Pharmaceuticals	Cayman Chemical Company
	Cygon (Dimethoate)	dimethyl {[(methylcarbamoyl)methyl]sulfanyl}(sulfanylidene)phosphonite	60-51-5	C5H12NO3PS2	228.9996	0.34	0.34	Biocides	Sigma-Aldrich
	Indomethacin	2-[1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-indol-3-yl]acetic acid	53-86-1	C19H16ClNO4	357.0768	2.31	0.5	Pharmaceuticals / TP	Cayman Chemical Company
	14-Hydroxyclarithromycin	(3R,4S,5S,6R,7R,9R,11R,12R,13S,14R)-6-{[(2S,3R,4S,6R)-4-(dimethylamino)-3-hydroxy-6-methyloxan-2-yl]oxy}-12,13-dihydroxy-4-{[(2R,4R,5S,6S)-5-hydroxy-4-methoxy-4,6-dimethyloxan-2-yl]oxy}-14-(1-hydroxyethyl)-7-methoxy-3,5,7,9,11,13-hexamethyl-1-oxacyclotetradecane-2,10-dione	110671-78-8	C38H69NO14	763.4718	-1.05	0.69	TP	CHEMOS

	Haloxyfop	(2R)-2-[4-[3-chloro-5-(trifluoromethyl)pyridin-2-yl]oxyphenoxy]propanoic acid	95977-29-0	C15H11ClF3NO4	361.0329	2.02	0.77	Pesticide	Sigma-Aldrich
	2-5-Dimethoxyaniline	2,5-dimethoxyaniline	102-56-7	C8H11NO2	153.0790	0.8	0.83	Pharmaceuticals	Sigma-Aldrich
	Venlafaxine	1-[2-(dimethylamino)-1-(4-methoxyphenyl)ethyl]cyclohexan-1-ol	93413-69-5	C17H27NO2	277.2042	-0.62	0.84	Pharmaceutical	Sigma-Aldrich
	Acetaminophen	N-(4-hydroxyphenyl)acetamide	103-90-2	C8H9NO2	151.0633	0.91	0.91	TP	Sigma-Aldrich
	Ipral	5-[(3,4,5-trimethoxyphenyl)methyl]pyrimidine-2,4-diamine	738-70-5	C14H18N4O3	290.1379	-0.11	0.92	Uncategorized	Sigma-Aldrich
	Acifluorfen	5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoic acid	50594-66-6	C14H7ClF3NO5	360.9965	1.27	1.03	Pesticide	Fluka
	Chloridazon	5-amino-4-chloro-2-phenyl-2,3-dihydropyridazin-3-one	1698-60-8	C10H8ClN3O	221.0356	1.11	1.11	Pesticide	Sigma-Aldrich
	Primidone	5-Ethyl-5-phenylperhydropyrimidine-4,6-dione	125-33-7	C12H14N2O2	218.1055	1.12	1.12	Pharmaceutical	Sigma-Aldrich
	Norfluoxetine	1-(3-amino-1-phenylpropoxy)-4-(trifluoromethyl)benzene	83891-03-6	C16H16F3NO	295.1184	0.72	1.16	Pharmaceutical	Sigma-Aldrich
	Triethylphosphate	Phosphoric acid, triethyl ester	78-40-0	C6H15O4P	182.0708	1.18	1.18	Pharmaceuticals / TP	Sigma-Aldrich
	Dapsone	4-(4-aminobenzenesulfonyl)aniline	80-08-0	C12H12N2O2S	248.0619	1.27	1.27	Pharmaceutical	Sigma-Aldrich
	O-Phenetidine	2-ethoxyaniline	94-70-2	C8H11NO	137.0841	1.24	1.34	REACH-chemical	Sigma-Aldrich
	P-Phenetidine	4-ethoxyaniline	156-43-4	C8H11NO	137.0841	0.98	1.34	Pharmaceuticals	Sigma-Aldrich
	1-2-Benzisothiazol-3(2H)-one	2,3-dihydro-1,2-benzothiazol-3-one	2634-33-5	C7H5NOS	151.0092	1.36	1.36	Biocides/ TP	Sigma-Aldrich
	Pelargonic acid	nonanoic acid	112-05-0	C9H18O2	158.1307	2.94	1.37	Pharmaceuticals / Biocides	Sigma-Aldrich
	Diclofenac	2-[2-(2,6-Dichlorophenyl)aminophenyl]acetic acid	15307-86-5	C14H11Cl2NO2	295.0167	3.21	1.37	Pharmaceutical	Sigma-Aldrich
	6-Methoxy-m-toluidine	2-methoxy-5-methylaniline	120-71-8	C8H11NO	137.0841	1.35	1.5	Pharmaceuticals	Sigma-Aldrich
	Fluoxetine	Benzenepropanamine, N-methyl-gamma-(4-(trifluoromethyl)phenoxy)-	54910-89-3	C17H18F3NO	309.1340	0.94	1.5	Pharmaceutical	Sigma-Aldrich
	Carboxin	2-methyl-N-phenyl-5,6-dihydro-1,4-oxathiine-3-carboxamide	5234-68-4	C12H13NO2S	235.0667	1.51	1.51	Pesticide	Dr. Ehrenstorfer
	Azamethiphos	dimethyl [({6-chloro-2-oxo-2H,3H-[1,3]oxazolo[4,5-b]pyridin-3-yl}methyl)sulfanyl]phosphonate	35575-96-3	C9H10ClN2O5PS	323.9737	1.52	1.52	Biocides	Fluka
	Hexahydro-4-methylphthalic anhydride	5-methyl-octahydro-2-benzofuran-1,3-dione	19438-60-9	C9H12O3	168.0786	1.53	1.53	Pharmaceuticals	Sigma-Aldrich

	Chlorsulfuron	1-(2-Chlorophenylsulfonyl)-3-(4-methoxy-6-methyl-1,3,5-triazin-2-yl)-urea	64902-72-3	C12H12ClN5O4S	357.0299	1.67	1.56	Transformation Products	Sigma-Aldrich
	Bayrepel	butan-2-yl 2-(2-hydroxyethyl)piperidine-1-carboxylate	119515-38-7	C12H23NO3	229.1678	1.61	1.61	Biocides / TP	Dr. Ehrenstorfer
	Carbetamide	(1S)-1-(ethylcarbamoyl)ethyl N-phenylcarbamate	16118-49-3	C12H16N2O3	236.1161	1.65	1.65	Pesticide	Sigma-Aldrich
	Methylparaben	4-hydroxybenzoic acid methyl ester	99-76-3	C8H8O3	152.0473	1.67	1.66	Pharmaceuticals / Biocides / TP	Sigma-Aldrich
	Ibuprofen	2-[4-(2-methylpropyl)phenyl]propanoic acid	15687-27-1	C13H18O2	206.1307	3.46	1.71	Pharmaceutical	Sigma-Aldrich
	Hymecromone	7-hydroxy-4-methyl-2H-chromen-2-one	90-33-5	C10H8O3	176.0473	1.78	1.72	Transformation Products	Sigma-Aldrich
	Diphenhydramine	[2-(diphenylmethoxy)ethyl]dimethylamine	58-73-1	C17H21NO	255.1623	0.31	1.79	TP	Sigma-Aldrich
	Oxadixyl	N-(2,6-dimethylphenyl)-2-methoxy-N-(2-oxo-3-oxazolidinyl)acetamide	77732-09-3	C14H18N2O4	278.1267	1.79	1.79	Pesticide	Fluka
	(+)-Catechin	(2R,3S)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-2H-1-benzopyran-3,5,7-triol	154-23-4	C15H14O6	290.0790	1.8	1.79	Pharmaceutical	Sigma-Aldrich
	Malathion	1,4-diethyl 2-{[dimethoxy(sulfanylidene)-λ^5-phosphanyl]sulfanyl}butanedioate	121-75-5	C10H19O6PS2	330.0361	1.86	1.86	Pesticide	Sigma-Aldrich
	Monuron	1-(4-chlorophenyl)-3,3-dimethylurea	150-68-5	C9H11ClN2O	198.0560	1.93	1.93	Pesticide	Sigma-Aldrich
	Metribuzin	4-amino-6-tert-butyl-3-(methylsulfanyl)-4,5-dihydro-1,2,4-triazin-5-one	21087-64-9	C8H14N4OS	214.0888	1.96	1.96	Pesticide	Sigma-Aldrich
Non-polar	2,6-Dichlorbenzamide	2,6-dichlorobenzamide	2008-58-4	C7H5Cl2NO	188.9748	2.03	2.03	Pesticides / TP	Sigma-Aldrich
	Carbofuran	2,2-dimethyl-2,3-dihydro-1-benzofuran-7-yl N-methylcarbamate	1563-66-2	C12H15NO3	221.1052	2.05	2.05	Pesticide	Fluka
	TCEP	Phosphoric acid, tris(2-chloroethyl) ester	115-96-8	C6H12Cl3O4P	283.9539	2.11	2.11	PCP	Sigma-Aldrich
	Amitriptyline	1-Propanamine, 3-(10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5-ylidene)-N,N-dimethyl-	50-48-6	C20H23N	277.1830	1.33	2.12	Pharmaceutical	Sigma-Aldrich
	Dilantin	5,5-diphenylimidazolidine-2,4-dione	57-41-0	C15H12N2O2	252.0899	2.15	2.15	Pharmaceutical	Fluka
	Atrazine	6-chloro-2-N-ethyl-4-N-(propan-2-yl)-1,3,5-triazine-2,4-diamine	1912-24-9	C8H14ClN5	215.0938	2.19	2.2	Pesticide	Sigma-Aldrich
	Metobromuron	1-(4-bromophenyl)-3-methoxy-3-methylurea	3060-89-7	C9H11BrN2O2	258.0004	2.24	2.24	Pesticide	Sigma-Aldrich
	Molinate	azepan-1-yl(ethylsulfanyl)methanone	2212-67-1	C9H17NOS	187.1031	2.34	2.34	Pesticide	Sigma-Aldrich
	Chlorotoluron	1-(2-chloro-4-methylphenyl)-3-methylurea	15545-48-9	C10H13ClN2O	212.0716	2.44	2.44	Pesticide	Sigma-Aldrich

	N,N-Diethyl-meta-toluamide	N,N-Diethyl-3-methylbenzamide	134-62-3	$C_{12}H_{17}NO$	191.1310	2.5	2.5	Biocides / TP	Sigma-Aldrich
	Propylparaben	4-Hydroxybenzoic acid propyl ester	94-13-3	$C_{10}H_{12}O_3$	180.0786	2.55	2.54	PCP	Fluka
	Valsartan	3-methyl-2-[N-({4-[2-(2H-1,2,3,4-tetrazol-5-yl)phenyl]phenyl}methyl)pentanamido]butanoic acid	137862-53-4	$C_{24}H_{29}N_5O_3$	435.2270	4.54	2.54	Uncategorized	Sigma-Aldrich
	Dimethyl sebacate	1,10-dimethyl decanedioate	106-79-6	$C_{12}H_{22}O_4$	230.1518	2.56	2.56	Pharmaceuticals	Sigma-Aldrich
	2-Ethyl-N-(2-ethylhexyl)-1-hexanamine	bis(2-ethylhexyl)amine	106-20-7	$C_{16}H_{35}N$	241.2770	2.61	2.67	REACH-chemical	Sigma-Aldrich
	Estriol	13-Methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthrene-3,16,17-triol	50-27-1	$C_{18}H_{24}O_3$	288.1725	2.67	2.67	Pharmaceutical	Sigma-Aldrich
	Pseudocumidine	2,4,5-trimethylaniline	137-17-7	$C_9H_{13}N$	135.1048	2.4	2.68	Uncategorized	Sigma-Aldrich
	Linuron	3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea	330-55-2	$C_9H_{10}Cl_2N_2O_2$	248.0119	2.68	2.68	Pesticide	Sigma-Aldrich
	Budesonide	(1S,2S,4R,6R,8S,9S,11S,12S,13R)-11-hydroxy-8-(2-hydroxyacetyl)-9,13-dimethyl-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-16-one	51333-22-3	$C_{25}H_{34}O_6$	430.2355	2.73	2.73	Pharmaceuticals	Sigma-Aldrich
	Carbamazepine	2-azatricyclo[9.4.0.0^{3},^{8}]pentadeca-1(15),3,5,7,9,11,13-heptaene-2-carboxamide	298-46-4	$C_{15}H_{12}N_2O$	236.0950	2.77	2.77	Pharmaceutical	Sigma-Aldrich
	Chlorbromuron	1-(4-bromo-3-chlorophenyl)-3-methoxy-3-methylurea	13360-45-7	$C_9H_{10}BrClN_2O_2$	291.9614	2.85	2.85	Pesticides	Dr. Ehrenstorfer
	Diphenamide	N,N-dimethyl-2,2-diphenylacetamide	957-51-7	$C_{16}H_{17}NO$	239.1310	2.93	2.93	TP	Sigma-Aldrich
	Metazachlor	2-chloro-N-(2,6-dimethylphenyl)-N-(1H-pyrazol-1-ylmethyl)acetamide	67129-08-2	$C_{14}H_{16}ClN_3O$	277.0982	2.98	2.98	Biocides / Pesticides / TP	Sigma-Aldrich
	Diethofencarb	propan-2-yl N-(3,4-diethoxyphenyl)carbamate	87130-20-9	$C_{14}H_{21}NO_4$	267.1471	3	3	TP	Sigma-Aldrich
	Butylated Hydroxyanisole	2-tert-butyl-4-methoxyphenol	25013-16-5	$C_{11}H_{16}O_2$	180.1150	3.06	3.06	Uncategorized	Sigma-Aldrich
	Chlorpropham	propan-2-yl N-(3-chlorophenyl)carbamate	101-21-3	$C_{10}H_{12}ClNO_2$	213.0557	3.21	3.21	Biocides	Sigma-Aldrich
	Flufenacet	N-(4-fluorophenyl)-N-(propan-2-yl)-2-{[5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl]oxy}acetamide	142459-58-3	$C_{14}H_{13}F_4N_3O_2S$	363.0665	3.22	3.22	Pesticide	Sigma-Aldrich
	1-4-Dioxacyclohexadecane-5-16-dione	1,4-dioxacyclohexadecane-5,16-dione	54982-83-1	$C_{14}H_{24}O_4$	256.1675	3.28	3.28	Pharmaceuticals	Sigma-Aldrich
	Ethyl [2-(4-phenoxyphenoxy)ethyl]carbamate (Fenoxycarb)	ethyl N-[2-(4-phenoxyphenoxy)ethyl]carbamate	79127-80-3	$C_{17}H_{19}NO_4$	301.1314	3.31	3.31	Uncategorized	Fluka

	Myristic acid	tetradecanoic acid	544-63-8	C14H28O2	228.2089	5.04	3.33	Biocides	Fluka
	Benzophenone-3 (Oxybenzone)	2-Hydroxy-4-methoxybenzophenone	131-57-7	C14H12O3	228.0786	3.62	3.36	TP	Sigma-Aldrich
	TCPP	tris(1-chloropropan-2-yl) phosphate	13674-84-5	C9H18Cl3O4P	326.0008	3.36	3.36	Flame retardant	Sigma-Aldrich
	Testosterone	(1S,2R,10R,11S,14S,15S)-14-hydroxy-2,15-dimethyltetracyclo[8.7.0.0^{2,7}.0^{11,15}]heptadec-6-en-5-one	58-22-0	C19H28O2	288.2089	3.37	3.37	NOM	Fluka
	Benzophenone	diphenylmethanone	119-61-9	C13H10O	182.0732	3.43	3.43	PCP	Sigma-Aldrich
	Metolachlor	2-chloro-N-(2-ethyl-6-methylphenyl)-N-(1-methoxypropan-2-yl)acetamide	51218-45-2	C15H22ClNO2	283.1339	3.45	3.45	Pesticide	Sigma-Aldrich
	Alachlor	2-chloro-N-(2,6-diethylphenyl)-N-(methoxymethyl)acetamide	15972-60-8	C14H20ClNO2	269.1183	3.59	3.59	Pesticide	Fluka
	Metconazole	5-[(4-chlorophenyl)methyl]-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentan-1-ol	125116-23-6	C17H22ClN3O	319.1451	3.59	3.59	Pesticide	Sigma-Aldrich
	Spironolacton	S-[(7R,8R,9S,10R,13S,14S,17R)-10,13-dimethyl-3,5'-dioxospiro[2,6,7,8,9,11,12,14,15,16-decahydro-1H-cyclopenta[a]phenanthrene-17,2'-oxolane]-7-yl] ethanethioate	52-01-7	C24H32O4S	416.2021	3.64	3.64	Pharmaceuticals / TP	Sigma-Aldrich
	17-Beta-Estradiol	13-Methyl-6,7,8,9,11,12,14,15,16,17-decahydrocyclopenta[a]phenanthrene-3,17-diol	50-28-2	C18H24O2	272.1776	3.75	3.75	TP	Sigma-Aldrich
	Acetyl Cedrene	1-{2,6,6,8-tetramethyltricyclo[5.3.1.$0^{1,5}$]undec-8-en-9-yl}ethan-1-one	32388-55-9	C17H26O	246.1984	3.87	3.87	TP	Sigma-Aldrich
	Ethinylestradiol	(1S,10R,11S,14R,15S)-14-ethynyl-15-methyltetracyclo[8.7.0.0^{2,7}.0^{11,15}]heptadeca-2(7),3,5-triene-5,14-diol	57-63-6	C20H24O2	296.1776	3.9	3.9	Pharmaceuticals / Biocides /	Sigma-Aldrich
	Bisphenol A	4-[2-(4-hydroxyphenyl)propan-2-yl]phenol	80-05-7	C15H16O2	228.1150	4.04	4.04	Uncategorized	Aldrich
	Prosulfocarb	N,N-dipropyl(benzylsulfanyl)formamide	52888-80-9	C14H21NOS	251.1344	4.17	4.17	Pesticide	Sigma-Aldrich
	Diazinon	ethyl 6-methyl-2-(propan-2-yl)pyrimidin-4-yl ethoxy(sulfanylidene)phosphonite	333-41-5	C12H21N2O3PS	304.1011	4.13	4.19	Pesticide	Dr. Ehrenstorfer
	TDCPP	tris(1,3-dichloropropan-2-yl) phosphate	13674-87-8	C9H15Cl6O4P	427.8839	4.28	4.28	Flame retardant	Sigma-Aldrich
	Chlorfenvinphos	(Z)-2-chloro-1-(2,4-dichlorophenyl)ethenyl diethyl phosphate	470-90-6	C12H14Cl3O4P	357.9695	4.3	4.3	Pesticide	Sigma-Aldrich
	Estrone	3-Hydroxy-13-methyl-7,8,9,11,12,14,15,16-octahydro-6H-cyclopenta[a]phenanthren-17-one	53-16-7	C18H22O2	270.1620	4.31	4.31	TP	Sigma-Aldrich
	Picoxystrobin	methyl (E)-3-methoxy-2-[2-[[6-(trifluoromethyl)pyridin-2-yl]oxymethyl]phenyl]prop-2-enoate	117428-22-5	C18H16F3NO4	367.1031	4.31	4.31	Pesticide	Sigma-Aldrich

	Linoleic acid	(9Z,12Z)-octadeca-9,12-dienoic acid	60-33-3	C18H32O2	280.2402	6.12	4.42	Uncategorized	Sigma-Aldrich
	Flurtamone	5-(methylamino)-2-phenyl-4-[3-(trifluoromethyl)phenyl]-2,3-dihydrofuran-3-one	96525-23-4	C18H14F3NO2	333.0977	4.64	4.64	Pesticide	Sigma-Aldrich
	Pyraclostrobin	methyl N-[2-({[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxy}methyl)phenyl]-N-methoxycarbamate	175013-18-0	C19H18ClN3O4	387.0986	4.7	4.7	Pesticide	Sigma-Aldrich
	Profenofos	4-bromo-2-chlorophenyl ethyl (propylsulfanyl)phosphonate	41198-08-7	C11H15BrClO3PS	371.9351	4.88	4.88	Pharmaceuticals / TP	Fluka
	Triclosan	5-Chloro-2-(2,4-dichlorophenoxy)-phenol	3380-34-5	C12H7Cl3O2	287.9512	4.98	4.9	Pesticide	Fluka
	Boscalid	2-chloro-N-[2-(4-chlorophenyl)phenyl]pyridine-3-carboxamide	188425-85-6	C18H12Cl2N2O	342.0327	4.92	4.92	Pesticide	Sigma-Aldrich
	Triclocarban	3-(4-chlorophenyl)-1-(3,4-dichlorophenyl)urea	101-20-2	C13H9Cl3N2O	313.9780	4.93	4.93	PCP	Sigma-Aldrich
	Quinoxyfen	5,7-dichloro-4-(4-fluorophenoxy)quinoline	124495-18-7	C15H8Cl2FNO	306.9967	4.95	4.98	Pesticide	Sigma-Aldrich
	Stearic acid	octadecanoic acid	57-11-4	C18H36O2	284.2715	6.82	5.11	Pharmaceuticals	Sigma-Aldrich
	Fenofibrate	propan-2-yl 2-[4-(4-chlorobenzoyl)phenoxy]-2-methylpropanoate	49562-28-9	C20H21ClO4	360.1128	5.28	5.28	Pharmaceutical	Sigma-Aldrich
	Fenazaquin	4-[2-(4-tert-butylphenyl)ethoxy]quinazoline	120928-09-8	C20H22N2O	306.1732	5.42	5.42	Pesticide	Fluka
	4-(7-Methyloctyl)phenol	4-(7-methyloctyl)phenol	84852-15-3	C15H24O	220.1827	5.58	5.58	Pharmaceuticals / REACH-chemical	Supelco
	Dibenzothiazyl disulfide	2-(1,3-benzothiazol-2-yldisulfanyl)-1,3-benzothiazole	120-78-5	C14H8N2S4	331.9570	6.22	6.22	Pharmaceuticals	Sigma-Aldrich
	6-6'-Di-tert-butyl-4-4'-thiodi-m-cresol	2-tert-butyl-4-[(5-tert-butyl-4-hydroxy-2-methylphenyl)sulfanyl]-5-methylphenol	96-69-5	C22H30O2S	358.1967	7.67	7.67	Pharmaceuticals	TCI

Table S- 8: Chromatographic conditions for the RPLC-HILIC serial coupling

	Binary pump 1				Binary pump 2				Isocratic pump		
Solvent A	10 mM ammonium acetate in water/acetonitrile (90/10, v/v)			Solvent C	acetonitrile			Solvent E	reference solution		
Solvent B	10 mM ammonium acetate in water/acetonitrile (10/90, v/v)			Solvent D	water						
Gradient	Time [min]	Flow rate [mL/min]	B%		Time [min]	Flow rate [mL/min]	D%		Time [min]	Flow rate [mL/min]	E%
	0	0.05	0		0	0.4	0		0	0.05	100
	7	0.05	0		6	0.4	0		58	0.05	100
	12	0.05	50		13	0.4	40				
	13	0.1	50		32	0.4	40				
	22	0.1	100		33	0.8	0				
	32	0.1	100		53	0.8	0				
	33	0.1	0		54	0.4	0				
	53	0.1	0		58	0.4	0				
	54	0.05	0								
	58	0.05	0								

Table S- 9: Chromatographic conditions for the SFC system

	Binary pump				Isocratic pump		
Solvent A	Carbon dioxide			Solvent F	reference solution		
Solvent B	20 mM ammonium acetate in methanol						
Gradient	Time [min]	Flow rate [mL/min]	B%		Time [min]	Flow rate [mL/min]	F%
	0	1.5	5		0	0.2	100
	2	1.5	5		25	0.2	100
	15	1.5	40				
	17	1.5	40				
	18	1.5	5				
	25	1.5	5				

Table S- 10: Jet-Stream ESI source parameters, used for the hyphenation of RPLC-HILIC and SFC with the ESI-TOF-MS. ESI parameters were identical for positive and negative ionization mode (except capillary voltage, which was used as positive voltage in negative ESI mode). Nitrogen was used for all gas flows.

	Gas temperature [°C]	Gas flow [L/min]	Nebulizer gas pressure [psi]	Sheath gas temperature [°C]	Sheath gas flow [L/min]	Capillary voltage [kilo volts]	Fragmentor voltage [volts]
RPLC-HILIC	325	10	45	325	7.5	- 3.0	100
SFC	275	5	45	275	6	- 4.0	100

Table S- 11: Retention times (RT) and relative standard deviations (RSD) of RTs from RPLC-HILIC/TOF-MS intraday and interday measurements of all standard compounds. All standards were injected three times in intra- and interday measurements and each ESI ionization mode. Ionization modes in which individual compounds could be detected are noted as "p" for positive and "n" for negative, or both "n/p". Overall RT and RSD include all intra- and interday results. RT of extended equilibration were derived from measurements with extended equilibration in order to investigate the equilibration state of the HILIC column. Table is sorted by log D (pH 7)

					ESI detection mode	RPLC-HILIC/TOF-MS intraday		RPLC-HILIC /TOF-MS interday		RPLC-HILIC/TOF-MS overall		RT extended equilibration	Deviation from overall RT [%]
	Compound name	**Empirical Formula**	**Monoisotopic Mass**	**log D (pH 7)**		**Mean RT [min]**	**RSD [%]**	**Mean RT [min]**	**RSD [%]**	**RT [min]**	**RSD [%]**		
Very polar	N,N-Bis(3-aminopropyl)methylamine	C7H19N3	145.1579	-7.71	p	11.42	0.24	11.51	0.19	11.47	0.48		
	Glyphosate	C3H8NO5P	169.0140	-6.9	n/p	13.19	0.78	12.88	0.53	13.04	1.37		
	1,3,4,5-Tetrahydroxycyclohexanecarboxylic acid	C7H12O6	192.0634	-5.93	n/p	11.98	0.66	11.82	0.25	11.90	0.83	11.79	0.93
	Cystine	C6H12N2O4S2	240.0238	-5.9	n/p	13.87	0.09	13.83	0.10	13.85	0.18		
	Glutamic acid	C5H9NO4	147.0532	-5.86	n/p	12.37	0.43	12.27	0.12	12.32	0.51		
	Moroxydine	C6H13N5O	171.1120	-5.43									
	Aspartic acid	C4H7NO4	133.0375	-5.37	n/p	12.40	0.54	12.31	0.15	12.35	0.54		
	Clavulanate	C8H9NO5	199.0481	-4.82	n	6.83	0.64	6.69	0.30	6.76	1.11		
	Oxaceprol	C7H11NO4	173.0688	-4.74	n/p	11.20	0.83	11.01	0.15	11.10	1.03		
	Maleic acid	C4H4O4	116.0110	-4.55	n	5.35	0.51	5.26	0.17	5.30	0.93		
	Tetracycline	C22H24N2O8	444.1533	-4.33	n/p	32.95	1.29	32.82	0.96	32.88	1.16		
	L-Asparagine	C4H8N2O3	132.0535	-4.3	n/p	13.49	0.20	13.44	0.19	13.47	0.26		
	Cefuroxime	C16H16N4O8S	424.0689	-4.26	n/p	17.81	0.59	17.64	1.83	17.73	1.43		
	Acetylcholine	C7H16NO2	146.1176	-4.22	p	7.85	0.21	7.75	0.44	7.80	0.74	7.8	0.01
	Acamprosate	C5H11NO4S	181.0409	-4.1	n	7.24	0.79	7.11	0.33	7.18	1.11	7.04	1.96
	Aminomethyl propanediol	C4H11NO2	105.0790	-4.04	p	17.41	1.70	17.89	0.23	17.65	1.81		
	Miglitol	C8H17NO5	207.1107	-3.89	n/p	11.59	0.19	11.55	0.06	11.57	0.25		
	Diethanolamine	C4H11NO2	105.0790	-3.79	p	19.71	1.49	20.13	0.18	19.92	1.49		
	Phenformin	C10H15N5	205.1327	-3.76	n/p	25.97	0.83	26.21	0.48	26.09	0.82		
	Betaine	C5H11NO2	117.0790	-3.72	n/p	11.75	0.17	11.67	0.12	11.71	0.39	11.60	0.91

	2-(2-Aminoethoxy)ethanol	C4H11NO2	105.0790	-3.71	p	19.75	1.37	20.29	0.06	20.02	1.64	20.17	0.76
	Histidine	C6H9N3O2	155.0695	-3.7	p	15.41	1.62	15.84	0.56	15.62	1.82		
	Metformin	C4H11N5	129.1014	-3.66	p	15.53	2.92	16.42	0.84	15.98	3.48		
	1H-Imidazole-1-propylamine	C6H11N3	125.0953	-3.65	n	5.94	0.23	5.92	0.05	5.93	0.21		
	1-(3'-Carboxypropyl)-3-7-dimethylxanthin	C11H14N4O4	266.1015	-3.6	n/p	7.81	1.25	7.65	0.21	7.73	1.38		
	Tenofovir	C9H14N5O4P	287.0783	-3.49	n	12.03	0.40	11.94	0.23	11.99	0.52		
	Threonine	C4H9NO3	119.0582	-3.47	n/p	13.13	0.37	13.01	0.19	13.07	0.54		
	Aminomethylpropanol	C4H11NO	89.0841	-3.35	p	17.86	1.30	18.41	0.22	18.13	1.78		
	Fructose	C6H12O6	180.0634	-3.27	n	11.02	0.15	10.93	0.32	10.97	0.48		
	Methylscopolamine	C18H24NO4	318.1700	-3.27	p	24.36	0.76	25.00	0.28	24.68	1.42		
	2-Ethylaminoethanol	C4H11NO	89.0841	-3.26	p	21.44	1.59	22.10	0.15	21.77	1.87	21.86	0.39
	1,1'-Iminodipropan-2-Ol	C6H15NO2	133.1103	-3.21	p	15.04	1.75	15.50	0.15	15.27	1.94	15.39	0.76
	Sarcosine	C3H7NO2	89.0477	-3.19	p	6.36	0.45	6.39	0.26	6.38	0.44	6.40	0.39
	Glycine	C2H5NO2	75.0320	-3.18	p	13.36	0.05	13.34	0.06	13.35	0.11		
	1-(N,N-Bis(2-hydroxyethyl)amino)propan-2-ol	C7H17NO3	163.1208	-3.18	p	9.14	0.13	9.12	0.23	9.13	0.22		
	beta-Alanine	C3H7NO2	89.0477	-3.17	p	13.49	0.15	13.45	0.07	13.47	0.18		
	Acetyllysine	C8H16N2O3	188.1161	-3.15	n	12.61	0.05	12.56	0.16	12.58	0.22		
	Lisinopril	C21H31N3O5	405.2264	-3.13	n/p	12.59	0.15	12.51	0.14	12.55	0.34		
	Chlortetracycline	C22H23ClN2O8	478.1143	-3.09	n/p	23.75	0.40	23.61	0.11	23.68	0.42		
	Acesulfame	C4H5NO4S	162.9939	-3.06	n	7.99	0.71	7.95	0.55	7.97	0.68		
	3-Ethoxypropylamine	C5H13NO	103.0997	-3.04	p	11.72	0.30	12.00	1.58	11.86	1.65	11.89	0.23
	Famotidine	C8H15N7O2S3	337.0449	-3.04	n/p	7.31	0.47	7.28	0.50	7.30	0.53		
	Trans-4-aminocyclohexan-1-ol	C6H13NO	115.0997	-3	p	20.46	0.97	21.09	0.21	20.77	1.68		
	Glucose	C6H12O6	180.0634	-2.93	n/p	12.13	0.19	12.09	0.25	12.11	0.28	12.04	0.59
	Gamma-aminobutyric acid	C4H9NO2	103.0633	-2.89	p	13.69	0.11	13.67	0.11	13.68	0.14		
	Triisopropanolamine	C9H21NO3	191.1521	-2.88	n/p	6.95	0.37	6.95	0.34	6.95	0.36		
	L-Alanine	C3H7NO2	89.0477	-2.84	p	13.00	0.01	12.94	0.12	12.97	0.27		

	2-Pyrrolidin-1-ylethanol	C6H13NO	115.0997	-2.69	p	23.33	1.75	24.21	0.40	23.77	2.22		
	Dexrazoxane	C11H16N4O4	268.1172	-2.65	p	6.15	0.38	6.10	0.14	6.13	0.52		
	Taurine	C2H7NO3S	125.0147	-2.62	n/p	12.66	0.06	12.61	0.08	12.64	0.20		
	Diethylamine	C4H11N	73.0891	-2.56	p	8.17	0.12	8.02	0.17	8.10	0.95		
	Ectoine	C6H10N2O2	142.0742	-2.53	n/p	12.75	0.25	12.68	0.13	12.71	0.32	12.64	0.57
Polar	Sotalol	C12H20N2O3S	272.1195	-2.47	n/p	10.75	0.41	10.78	0.14	10.76	0.34		
	Amoxicillin	C16H19N3O5S	365.1045	-2.43	n	7.99	0.69	7.86	0.28	7.93	0.99		
	2-[2-(Dimethylamino)ethoxy]ethanol	C6H15NO2	133.1103	-2.41	p	18.56	1.57	19.15	0.09	18.86	1.90		
	2-Butylaminoethanol	C6H15NO	117.1154	-2.37	p	11.59	0.24	11.67	0.22	11.63	0.40	11.60	0.21
	Allantoin	C4H6N4O3	158.0440	-2.37	n	10.44	0.02	10.40	0.19	10.42	0.21		
	Piperidine	C5H11N	85.0891	-2.36	p	21.15	1.36	21.71	0.53	21.43	1.65		
	Guanylurea	C2H6N4O	102.0542	-2.36	n/p	14.53	1.46	14.68	0.32	14.61	1.18		
	L-Proline	C5H9NO2	115.0633	-2.34	n/p	12.25	0.10	12.18	0.25	12.21	0.37		
	2-(Dimethylamino)-2-methylpropan-1-ol	C6H15NO	117.1154	-2.34	p	19.02	2.00	19.59	0.52	19.31	2.07		
	2,2,6,6-Tetramethylpiperidin-4-ol	C9H19NO	157.1467	-2.34									
	D(-) Ribose	C5H10O5	150.0528	-2.3	n	8.21	0.31	8.13	0.08	8.17	0.50		
	Cefalexin	C16H17N3O4S	347.0940	-2.26	p	30.70	0.03	30.69	0.09	30.69	0.07		
	Minoxidil	C9H15N5O	209.1277	-2.25	p	23.48	0.03	23.52	0.05	23.50	0.09		
	L-Methionine	C5H11NO2S	149.0510	-2.19	n/p	11.31	0.11	11.26	0.07	11.29	0.25		
	Atenolol	C14H22N2O3	266.1630	-2.14	n/p	11.41	0.55	11.46	0.13	11.44	0.44	11.40	0.27
	Butylamine	C4H11N	73.0891	-2.11	p	11.45	0.30	11.56	0.29	11.50	0.54	11.50	0.00
	1,2,2,6,6-Pentamethylpiperidin-4-ol	C10H21NO	171.1623	-2.11									
	Adenosine	C10H13N5O4	267.0968	-2.1	n/p	7.33	0.26	7.30	0.44	7.31	0.41	7.29	0.38
	Vigabatrin	C6H11NO2	129.0790	-2.09	n	12.75	0.10	12.71	0.16	12.73	0.23		
	Melamine	C3H6N6	126.0654	-2.02	p	7.45	0.27	7.44	0.10	7.44	0.21		
	6-Amino-5-formamido-1-3-dimethyluracil	C7H10N4O3	198.0753	-2.02	n/p	6.80	0.59	6.74	0.55	6.77	0.69	6.84	0.98
	Valine	C5H11NO2	117.0790	-1.95	n/p	11.76	0.11	11.72	0.24	11.74	0.25		

	Butylscopolamine	C21H30NO4	360.2169	-1.94	p	27.43	0.19	27.65	0.39	27.54	0.51		
	Syringic acid	C9H10O5	198.0528	-1.93	n	6.93	0.51	6.84	0.09	6.89	0.75		
	Octopamine	C8H11NO2	153.0790	-1.92									
	N,N-Dimethylisopropylamine	C5H13N	87.1048	-1.9									
	Oxypurinol	C5H4N4O2	152.0334	-1.88	n/p	7.08	0.56	7.04	1.12	7.06	0.93	7.12	0.79
	Norfenefrine	C8H11NO2	153.0790	-1.88	n/p	14.68	1.69	14.91	0.40	14.79	1.45	14.80	0.06
	N-[3-(Dimethylamino)propyl]methacrylamide	C9H18N2O	170.1419	-1.85	p	25.18	0.07	25.19	0.10	25.18	0.09		
	Isopentylamine	C5H13N	87.1048	-1.82	p	10.88	0.38	10.97	0.22	10.92	0.51	10.92	0.03
	N,N'-Ethylenedi(diacetamide)	C6H12N2O2	144.0899	-1.78	n/p	6.25	0.54	6.24	0.66	6.25	0.60		
	Diisopropylamine	C6H15N	101.1204	-1.76	p	11.01	0.38	11.11	0.13	11.06	0.51		
	Dopamine	C8H11NO2	153.0790	-1.75	p	25.81	0.07	25.85	0.06	25.83	0.10		
	Panthenol	C9H19NO4	205.1314	-1.7	n/p	6.62	0.37	6.58	0.25	6.60	0.41		
	Cyanurodiamide (Atrazin-desethyl-desisopropyl-2-hydroxy)	C3H5N5O	127.0494	-1.69	p	10.89	0.15	10.85	0.08	10.87	0.22	10.77	0.88
	Dacarbazine	C6H10N6O	182.0916	-1.69	p	6.69	0.21	6.61	0.19	6.65	0.64		
	Piracetam	C6H10N2O2	142.0742	-1.68	p	6.32	0.11	6.26	0.31	6.29	0.48		
	Ethylenediaminetetraacetonitrile	C10H12N6	216.1123	-1.65	n/p	19.70	1.25	19.56	0.46	19.63	1.01		
	Dipropylamine	C6H15N	101.1204	-1.56	p	10.83	0.26	10.95	0.36	10.89	0.64		
	Tranexamic acid	C8H15NO2	157.1103	-1.55	n/p	13.99	0.08	14.10	1.23	14.04	0.95		
	4-Hydroxybenzenesulfonic acid	C6H6O4S	173.9987	-1.53	n	7.34	0.40	7.24	0.21	7.29	0.77		
	Tyramine	C8H11NO	137.0841	-1.52	p	14.28	0.71	14.43	0.87	14.36	0.96		
	L-Isoleucine	C6H13NO2	131.0946	-1.51	n/p	11.01	0.09	10.93	0.19	10.97	0.39		
	Acetamide MEA	C4H9NO2	103.0633	-1.5	n/p	6.18	0.27	6.16	0.16	6.17	0.25		
	L-Tyrosine	C9H11NO3	181.0739	-1.49	n/p	11.98	0.39	11.91	0.04	11.95	0.39		
	Etilefrine	C10H15NO2	181.1103	-1.42	n/p	11.47	0.37	11.51	0.16	11.49	0.35		
	Dimethylsulfoxide	C2H6OS	78.0139	-1.41	p	5.97	0.14	5.94	0.15	5.95	0.28	5.97	0.27
	N-(Hydroxymethyl)Acetamide	C3H7NO2	89.0477	-1.39	p	12.90	0.05	12.84	0.07	12.87	0.25		
	Phenylpyruvic acid	C9H8O3	164.0473	-1.39	n	6.10	0.62	6.12	0.40	6.11	0.54		

	Hydroxycarbamide	CH4N2O2	76.0273	-1.37	p	7.24	1.05	7.23	0.29	7.23	0.77		
	(2,4-Diaminopteridin-6-yl)yethanol	C7H8N6O	192.0760	-1.37	p	7.19	0.18	7.14	0.18	7.16	0.38		
	Urea	CH4N2O	60.0324	-1.36	p	7.04	0.41	6.98	0.13	7.01	0.48		
	Pregabalin	C8H17NO2	159.1259	-1.35	n/p	10.13	0.11	10.07	0.08	10.10	0.30		
	Tetraethylene glycol	C8H18O5	194.1154	-1.35	p	6.45	0.32	6.44	0.10	6.44	0.28		
	2-6-Dimethylmorpholine	C6H13NO	115.0997	-1.33	p	10.82	0.64	10.90	0.20	10.86	0.61		
	Gabapentin	C9H17NO2	171.1259	-1.27	n/p	10.04	0.31	10.13	1.09	10.08	0.94		
	Ferulic acid	C10H10O4	194.0579	-1.24	n	7.52	0.39	7.42	0.09	7.47	0.76		
	Atenolol acid (metoprolol acid)	C14H21NO4	267.1471	-1.24	n/p	12.53	0.33	12.46	0.08	12.49	0.37		
	Riboflavin	C17H20N4O6	376.1383	-1.23	n/p	7.81	0.32	7.77	0.11	7.79	0.35		
	Methacrylic Acid	C4H6O2	86.0368	-1.21	p	13.69	0.11	13.67	0.11	13.68	0.14		
	3-(2-Aminopropyl)phenol	C9H13NO	151.0997	-1.19	p	11.33	0.22	11.41	0.07	11.37	0.37		
	(R)-1-(4-methoxyphenyl)ethylamine	C9H13NO	151.0997	-1.19	p	14.06	1.35	14.31	0.11	14.18	1.30		
	Phenylalanine	C9H11NO2	165.0790	-1.19	n/p	10.57	0.10	10.53	0.21	10.55	0.25		
	Cytosine	C4H5N3O	111.0433	-1.15	n/p	8.14	0.44	8.10	1.31	8.12	1.01		
	4-Methylmorpholine 4-oxide	C5H11NO2	117.0790	-1.15	p	12.47	0.16	12.41	0.07	12.44	0.26		
	Tolazoline	C10H12N2	160.1000	-1.15	p	10.07	0.21	10.16	0.51	10.11	0.60	10.10	0.12
	Methylurea	C2H6N2O	74.0480	-1.14	p	6.19	0.49	6.14	0.37	6.17	0.61		
	Cyanuric acid	C3H3N3O3	129.0174	-1.14	n	6.82	0.23	6.85	1.57	6.84	1.15		
	2-Morpholinoethanol	C6H13NO2	131.0946	-1.13	p	6.96	0.09	6.95	0.11	6.95	0.13		
	Tryptophan	C11H12N2O2	204.0899	-1.09	n/p	11.00	0.12	10.97	0.06	10.98	0.17		
	D-α-methylbenzylamine	C8H11N	121.0891	-1.04	p	13.98	0.92	14.18	0.08	14.08	0.96		
	L-α-Methylbenzylamine	C8H11N	121.0891	-1.04	p	9.48	0.84	9.59	0.24	9.53	0.84		
	Cyanoguanidine	C2H4N4	84.0436	-1.03									
	Perhydropyrimidin-2-one	C4H8N2O	100.0637	-1.03	p	6.84	0.29	6.77	0.21	6.81	0.53		
	Propylidynetrimethanol	C6H14O3	134.0943	-1.01	n	6.60	0.14	6.61	0.33	6.60	0.26		
	Ethyl glycinate	C4H9NO2	103.0633	-0.99	p	6.42	0.28	6.43	0.19	6.42	0.26	6.49	0.97
	Pyridoxine	C8H11NO3	169.0739	-0.97	n/p	6.79	0.46	6.75	0.52	6.77	0.56		
	Emtricitabine	C8H10FN3O3S	247.0427	-0.9	n/p	6.39	0.20	6.35	0.26	6.37	0.40		

	P-Coumaric acid	C9H8O3	164.0473	-0.89	n	7.52	0.36	7.42	0.23	7.47	0.73		
	N-Formylmorpholine	C5H9NO2	115.0633	-0.85	p	6.17	0.22	6.16	0.32	6.16	0.28		
	Vasotec	C20H28N2O5	376.1998	-0.85	n/p	24.38	0.31	24.40	0.04	24.39	0.22		
	2-Hydroxy-N,N-dimethyl-propanamide	C5H11NO2	117.0790	-0.83	n/p	6.26	0.82	6.22	0.94	6.24	0.94		
	N-(Hydroxymethyl)nicotinamide	C7H8N2O2	152.0586	-0.75	n/p	6.54	0.27	6.51	0.22	6.52	0.34		
	N-(2-Hydroxyethyl)prop-2-enamide	C5H9NO2	115.0633	-0.74	n/p	6.15	0.22	6.13	0.46	6.14	0.39	6.17	0.44
	1,4-Butinodiol	C4H6O2	86.0368	-0.73	p	6.42	0.28	6.43	0.19	6.42	0.26	6.49	0.97
	2-2'-Dimorpholinyldiethyl ether	C12H24N2O3	244.1787	-0.71	p	14.42	0.80	14.75	0.98	14.59	1.44		
	Lenalidomide	C13H13N3O3	259.0957	-0.71	p	15.33	1.18	15.31	0.14	15.32	0.85		
	Isoniazid	C6H7N3O	137.0589	-0.69	p	6.53	0.10	6.50	0.19	6.52	0.27		
	4-Methylmorpholine	C5H11NO	101.0841	-0.66	p	8.61	1.79	8.69	0.16	8.65	1.35		
	Methoxytriglycol	C7H16O4	164.1049	-0.66	p	6.23	0.18	6.23	0.08	6.23	0.15		
	1,3-Dimethylimidazolidin-2-one	C5H10N2O	114.0793	-0.64	p	6.10	0.12	6.11	0.20	6.10	0.18		
	Diatrizoate	C11H9I3N2O4	613.7697	-0.62	n/p	9.35	1.36	8.85	0.33	9.10	2.95		
	Arecoline	C8H13NO2	155.0946	-0.6	p	6.76	0.36	6.71	0.09	6.73	0.43	6.74	0.03
	2-Pyrrolidone	C4H7NO	85.0528	-0.58	p	6.32	0.23	6.24	1.52	6.28	1.28		
	Adenine	C5H5N5	135.0545	-0.58	p	7.03	0.12	6.98	0.15	7.01	0.43	7.04	0.43
	Asulam	C8H10N2O4S	230.0361	-0.58	n/p	6.32	0.60	6.26	0.30	6.29	0.66		
	Hydrochlorthiazide	C7H8ClN3O4S2	296.9645	-0.58	p	17.68	0.46	17.74	0.23	17.71	0.40		
	2-Methylimidazole	C4H6N2	82.0531	-0.55	p	7.42	0.45	7.45	0.10	7.43	0.39		
	Omethoate	C5H12NO4PS	213.0225	-0.55	p	6.23	0.22	6.24	0.05	6.23	0.17		
	Sucralose	C12H19Cl3O8	396.0146	-0.47	n/p	22.86	0.15	22.85	0.29	22.86	0.23		
	Iopromide	C18H24I3N3O8	790.8698	-0.44	n/p	8.58	0.92	8.68	1.10	8.63	1.16		
	2-Ethylhexylamine	C8H19N	129.1517	-0.42	p	27.49	0.16	27.87	0.20	27.68	0.72		
	Ethyl 2-oxopyrrolidine-1-acetate	C8H13NO3	171.0895	-0.38	p	17.50	0.40	17.68	0.21	17.59	0.60		
	Ritalinic Acid	C13H17NO2	219.1259	-0.36	n/p	8.41	0.34	8.36	0.28	8.38	0.44		
	Cimetidine	C10H16N6S	252.1157	-0.34	n/p	6.59	0.79	6.59	1.30	6.59	1.08	6.52	1.07
	2,2,6,6-Tetramethyl-4-piperidone	C9H17NO	155.1310	-0.32	p	6.55	0.33	6.56	0.10	6.55	0.25		
	Dorzolamide	C10H16N2O4S3	324.0272	-0.32	n/p	24.83	0.05	24.79	0.05	24.81	0.08		

	4-Methylimidazole	C4H6N2	82.0531	-0.3	p	6.87	0.12	6.89	0.19	6.88	0.19		
	3-Dimethylaminopropiononitrile	C5H10N2	98.0844	-0.3	p	6.22	0.11	6.20	0.11	6.21	0.16		
	Diethylene glycol ethyl ether	C6H14O3	134.0943	-0.26									
	1,2-Dimethylimidazole	C5H8N2	96.0687	-0.25	p	7.65	0.26	7.72	0.28	7.69	0.48		
	Methyl 3-aminocrotonate	C5H9NO2	115.0633	-0.24	n/p	6.36	0.28	6.34	0.26	6.35	0.30		
	6-Mercaptopurine	C5H4N4S	152.0157	-0.12	p	7.13	0.21	7.09	0.18	7.11	0.37		
	Candesartan	C24H20N6O3	440.1597	-0.12	n/p	23.27	0.06	23.26	0.05	23.26	0.07		
	2-Mercaptoethanol	C2H6OS	78.0139	-0.11	p	5.98	0.36	5.94	0.39	5.96	0.48		
	[2-(Acryloyloxy)ethyl]trimethylammonium	C8H15NO2	157.1103	-0.1	n/p	10.84	0.70	10.91	0.43	10.87	0.68	10.89	0.12
	1,4-Dioxane	C4H8O2	88.0524	-0.09	p	19.71	1.49	20.13	0.18	19.92	1.49		
	Hexanoic acid	C6H12O2	116.0837	-0.09	p	10.88	0.06	10.83	0.08	10.85	0.25		
	1-Methylimidazole	C4H6N2	82.0531	-0.07	p	6.30	0.14	6.32	0.14	6.31	0.22	6.37	0.91
	Tetraglyme	C10H22O5	222.1467	-0.06	p	18.52	0.23	18.78	0.46	18.65	0.77		
	Nicotinylalcohol	C6H7NO	109.0528	-0.01	p	6.15	0.14	6.14	0.17	6.15	0.17	6.19	0.63
	2-Phenyl-1H-benzimidazole-5-sulphonic acid	C13H10N2O3S	274.0412	0.09									
	Pipamperone	C21H30FN3O2	375.2322	0.13	n/p	26.90	0.21	26.93	0.28	26.91	0.26		
	Sulfamethoxazole	C10H11N3O3S	253.0521	0.14	n/p	17.47	0.30	17.52	0.52	17.50	0.45		
	Naproxen	C14H14O3	230.0943	0.25	n/p	24.56	0.11	24.55	0.07	24.56	0.09		
	Cygon (Dimethoate)	C5H12NO3PS2	228.9996	0.34	n/p	24.75	0.03	24.73	0.04	24.74	0.06		
	Indomethacin	C19H16ClNO4	357.0768	0.5	n/p	25.84	0.06	25.87	0.08	25.85	0.09		
	14-Hydroxyclarithromycin	C38H69NO14	763.4718	0.69	n/p	27.22	0.21	27.42	0.31	27.32	0.45		
	Haloxyfop	C15H11ClF3NO4	361.0329	0.77	n/p	25.55	0.05	25.58	0.09	25.57	0.10		
	2-5-Dimethoxyaniline	C8H11NO2	153.0790	0.83	p	25.37	0.05	25.34	0.00	25.36	0.07		
	Venlafaxine	C17H27NO2	277.2042	0.84	p	27.48	0.23	27.73	0.41	27.61	0.56		
	Acetaminophen	C8H9NO2	151.0633	0.91	p	27.62	0.03	27.61	0.11	27.62	0.08	27.71	0.32
	Ipral	C14H18N4O3	290.1379	0.92	n/p	24.16	0.07	24.15	0.10	24.15	0.09		
	Acifluorfen	C14H7ClF3NO5	360.9965	1.03	n/p	25.85	0.05	25.84	0.09	25.85	0.07	25.89	0.17

	Chloridazon	C10H8ClN3O	221.0356	1.11	n/p	24.46	0.06	24.46	0.07	24.46	0.06	24.69	0.93
	Primidone	C12H14N2O2	218.1055	1.12	n/p	23.76	0.11	23.72	0.11	23.74	0.14		
	Norfluoxetine	C16H16F3NO	295.1184	1.16	p	29.04	0.25	29.26	0.33	29.15	0.48		
	Triethylphosphate	C6H15O4P	182.0708	1.18	p	25.08	0.09	25.07	0.02	25.07	0.07		
	Dapsone	C12H12N2O2S	248.0619	1.27	p	24.66	0.03	24.60	0.08	24.63	0.15		
	O-Phenetidine	C8H11NO	137.0841	1.34									
	P-Phenetidine	C8H11NO	137.0841	1.34									
	1-2-Benzisothiazol-3(2H)-one	C7H5NOS	151.0092	1.36	n/p	25.66	0.06	25.65	0.11	25.65	0.09	25.77	0.47
	Pelargonic acid	C9H18O2	158.1307	1.37	n	26.32	0.08	26.30	0.05	26.31	0.07		
	Diclofenac	C14H11Cl2NO2	295.0167	1.37	n/p	25.67	0.08	25.67	0.08	25.67	0.08		
	6-Methoxy-m-toluidine	C8H11NO	137.0841	1.5	p	26.80	0.03	26.80	0.14	26.80	0.10	26.91	0.39
	Fluoxetine	C17H18F3NO	309.1340	1.5	n/p	29.36	0.22	29.47	0.30	29.41	0.32		
	Carboxin	C12H13NO2S	235.0667	1.51	n/p	27.81	0.04	27.81	0.06	27.81	0.05	27.87	0.23
	Azamethiphos	C9H10ClN2O5PS	323.9737	1.52	p	26.57	0.03	26.57	0.11	26.57	0.08	26.64	0.27
	Hexahydro-4-methylphthalic anhydride	C9H12O3	168.0786	1.53									
	Chlorsulfuron	C12H12ClN5O4S	357.0299	1.56	n/p	23.01	0.09	22.98	0.09	23.00	0.11	23.36	1.56
	Bayrepel	C12H23NO3	229.1678	1.61	p	27.64	0.06	27.65	0.06	27.65	0.06		
	Carbetamide	C12H16N2O3	236.1161	1.65	n/p	25.79	0.05	25.79	0.06	25.79	0.05		
	Methylparaben	C8H8O3	152.0473	1.66	n/p	25.39	0.07	25.41	0.05	25.40	0.07		
	Ibuprofen	C13H18O2	206.1307	1.71	n/p	26.15	0.07	26.20	0.11	26.18	0.13		
	Hymecromone	C10H8O3	176.0473	1.72	n/p	25.13	0.09	25.14	0.09	25.14	0.10		
	Diphenhydramine	C17H21NO	255.1623	1.79	p	28.16	0.17	28.32	0.36	28.24	0.40		
	Oxadixyl	C14H18N2O4	278.1267	1.79	p	26.29	0.07	26.30	0.07	26.30	0.07		
	(+)-Catechin	C15H14O6	290.0790	1.79	p	16.42	0.77	16.41	0.69	16.42	0.73		
	Malathion	C10H19O6PS2	330.0361	1.86	n/p	30.44	0.06	30.46	0.04	30.45	0.06		
	Monuron	C9H11ClN2O	198.0560	1.93	n/p	26.29	0.04	26.32	0.05	26.30	0.07		
	Metribuzin	C8H14N4OS	214.0888	1.96	n/p	26.81	0.07	26.84	0.07	26.82	0.10		
Non-polar	2,6-Dichlorbenzamide	C7H5Cl2NO	188.9748	2.03	p	20.41	0.53	20.20	0.37	20.31	0.68		

	Carbofuran	C12H15NO3	221.1052	2.05	p	27.12	0.03	27.13	0.08	27.12	0.06	27.19	0.25
	TCEP	C6H12Cl3O4P	283.9539	2.11	p	27.12	0.04	27.13	0.05	27.12	0.05		
	Amitriptyline	C20H23N	277.1830	2.12	p	29.94	0.25	30.14	0.41	30.04	0.47	30.12	0.28
	Dilantin	C15H12N2O2	252.0899	2.15	n/p	26.53	0.06	26.53	0.09	26.53	0.08		
	Atrazine	C8H14ClN5	215.0938	2.2	p	27.63	0.01	27.64	0.11	27.63	0.07	27.72	0.29
	Metobromuron	C9H11BrN2O2	258.0004	2.24	n/p	28.25	0.03	28.27	0.04	28.26	0.05		
	Molinate	C9H17NOS	187.1031	2.34	p	30.17	0.01	30.21	0.04	30.19	0.06		
	Chlorotoluron	C10H13ClN2O	212.0716	2.44	n/p	27.30	0.06	27.30	0.07	27.30	0.06	27.36	0.21
	N,N-Diethyl-meta-toluamide	C12H17NO	191.1310	2.5	p	27.69	0.04	27.69	0.08	27.69	0.06		
	Propylparaben	C10H12O3	180.0786	2.54	n/p	27.72	0.04	27.72	0.08	27.72	0.06		
	Valsartan	C24H29N5O3	435.2270	2.54	n/p	24.06	0.07	24.04	0.10	24.05	0.09		
	Dimethyl sebacate	C12H22O4	230.1518	2.56	p	30.58	0.02	30.58	0.08	30.58	0.06		
	2-Ethyl-N-(2-ethylhexyl)-1-hexanamine	C16H35N	241.2770	2.67	p	33.06	0.42	33.45	0.72	33.26	0.84	33.52	0.80
	Estriol	C18H24O3	288.1725	2.67	n/p	25.06	0.04	25.05	0.06	25.06	0.06		
	Pseudocumidine	C9H13N	135.1048	2.68	p	28.06	0.03	28.07	0.04	28.07	0.04		
	Linuron	C9H10Cl2N2O2	248.0119	2.68	n/p	29.40	0.03	29.42	0.03	29.41	0.05		
	Budesonide	C25H34O6	430.2355	2.73	n/p	28.30	0.04	28.30	0.09	28.30	0.07	28.32	0.08
	Carbamazepine	C15H12N2O	236.0950	2.77	p	26.41	0.00	26.41	0.11	26.41	0.08	26.47	0.23
	Chlorbromuron	C9H10BrClN2O2	291.9614	2.85	n/p	29.67	0.04	29.66	0.06	29.67	0.06	29.69	0.08
	Diphenamide	C16H17NO	239.1310	2.93	p	28.51	0.04	28.49	0.08	28.50	0.07		
	Metazachlor	C14H16ClN3O	277.0982	2.98	p	28.39	0.03	28.43	0.03	28.41	0.08		
	Diethofencarb	C14H21NO4	267.1471	3	n/p	29.27	0.05	29.27	0.07	29.27	0.06	29.31	0.12
	Butylated Hydroxyanisole	C11H16O2	180.1150	3.06	n	29.62	0.06	29.61	0.04	29.61	0.05		
	Chlorpropham	C10H12ClNO2	213.0557	3.21	n/p	30.28	0.03	30.26	0.08	30.27	0.07		
	Flufenacet	C14H13F4N3O2S	363.0665	3.22	p	30.64	0.02	30.63	0.05	30.63	0.04		
	1-4-Dioxacyclohexadecane-5-16-dione	C14H24O4	256.1675	3.28	p	28.16	0.17	28.31	0.40	28.23	0.40		
	Ethyl [2-(4-phenoxyphenoxy)ethyl]carbamate (Fenoxycarb)	C17H19NO4	301.1314	3.31	p	30.48	0.00	30.47	0.05	30.47	0.04		

	Myristic acid	C14H28O2	228.2089	3.33	p	29.09	0.13	29.27	0.32	29.18	0.40		
	Benzophenone-3 (Oxybenzone)	C14H12O3	228.0786	3.36	n/p	31.15	0.05	31.13	0.03	31.14	0.05		
	TCPP	C9H18Cl3O4P	326.0008	3.36	p	29.13	0.03	29.13	0.06	29.13	0.05		
	Testosterone	C19H28O2	288.2089	3.37	p	28.03	0.06	28.04	0.09	28.03	0.08		
	Benzophenone	C13H10O	182.0732	3.43	p	30.12	0.05	30.11	0.07	30.11	0.07	30.14	0.09
	Metolachlor	C15H22ClNO2	283.1339	3.45	p	30.85	0.02	30.86	0.04	30.86	0.04		
	Alachlor	C14H20ClNO2	269.1183	3.59	p	30.86	0.03	30.87	0.10	30.86	0.07	30.87	0.04
	Metconazole	C17H22ClN3O	319.1451	3.59	n/p	30.56	0.05	30.58	0.06	30.57	0.06		
	Spironolacton	C24H32O4S	416.2021	3.64	p	28.99	0.01	28.98	0.06	28.99	0.05		
	17-Beta-Estradiol	C18H24O2	272.1776	3.75	n/p	27.71	0.07	27.72	0.08	27.72	0.08	27.74	0.08
	Acetyl Cedrene	C17H26O	246.1984	3.87									
	Ethinylestradiol	C20H24O2	296.1776	3.9	n/p	28.30	0.02	28.29	0.08	28.30	0.06	28.34	0.15
	Bisphenol A	C15H16O2	228.1150	4.04	n	27.57	0.05	27.57	0.04	27.57	0.05		
	Prosulfocarb	C14H21NOS	251.1344	4.17	p	34.16	0.07	34.12	0.04	34.14	0.08		
	Diazinon	C12H21N2O3PS	304.1011	4.19	p	32.37	0.02	32.35	0.02	32.36	0.04		
	TDCPP	C9H15Cl6O4P	427.8839	4.28	n/p	31.06	0.05	31.04	0.03	31.05	0.05		
	Chlorfenvinphos	C12H14Cl3O4P	357.9695	4.3	p	31.15	0.04	31.13	0.08	31.14	0.07	31.17	0.09
	Estrone	C18H22O2	270.1620	4.31	n/p	28.70	0.03	28.70	0.04	28.70	0.04		
	Picoxystrobin	C18H16F3NO4	367.1031	4.31	p	31.38	0.05	31.36	0.04	31.37	0.06		
	Linoleic acid	C18H32O2	280.2402	4.42	n/p	37.03	0.01	37.02	0.02	37.02	0.01		
	Flurtamone	C18H14F3NO2	333.0977	4.64	n/p	28.96	0.05	28.97	0.07	28.96	0.07		
	Pyraclostrobin	C19H18ClN3O4	387.0986	4.7	p	32.25	0.09	32.23	0.04	32.24	0.08		
	Profenofos	C11H15BrClO3PS	371.9351	4.88	p	33.81	0.09	33.76	0.05	33.78	0.11		
	Triclosan	C12H7Cl3O2	287.9512	4.9	n	32.54	0.04	32.53	0.02	32.53	0.03		
	Boscalid	C18H12Cl2N2O	342.0327	4.92	n/p	29.82	0.05	29.81	0.09	29.81	0.07	29.84	0.09
	Triclocarban	C13H9Cl3N2O	313.9780	4.93	n/p	32.44	0.14	32.40	0.07	32.42	0.13		
	Quinoxyfen	C15H8Cl2FNO	306.9967	4.98	p	34.53	0.02	34.53	0.02	34.53	0.02		
	Stearic acid	C18H36O2	284.2715	5.11	p	36.89	0.17	36.87	0.16	36.88	0.17		
	Fenofibrate	C20H21ClO4	360.1128	5.28	p	35.27	0.04	35.24	0.06	35.26	0.07		

	Fenazaquin	$C_{20}H_{22}N_2O$	306.1732	5.42	p	35.77	0.04	35.74	0.06	35.75	0.07		
	4-(7-Methyloctyl)phenol	$C_{15}H_{24}O$	220.1827	5.58	p	30.18	0.05	30.18	0.02	30.18	0.04		
	Dibenzothiazyl disulfide	$C_{14}H_8N_2S_4$	331.9570	6.22	p	26.26	0.14	26.22	0.13	26.24	0.15		
	6-6'-Di-tert-butyl-4-4'-thiodi-m-cresol	$C_{22}H_{30}O_2S$	358.1967	7.67	n/p	34.96	0.11	34.92	0.05	34.94	0.10		

Appendix

Table S- 12: Retention times (RT) and relative standard deviations (RSD) of RTs from SFC/TOF-MS intraday and interday measurements of all standard compounds. All standards were injected three times in intra- and interday measurements and each ESI ionization mode. Ionization modes in which individual compounds could be detected are noted as "p" for positive and "n" for negative, or both "n/p". Overall RT and RSD include all intra- and interday results. The retention factor of retained compounds was calculated using a void time of 0.6 minutes. Table is sorted by log D (pH 5)

						ESI detection mode	SFC/TOF-MS intraday		SFC/TOF-MS interday		SFC/TOF-MS overall		Retention factor
	Compound name	**Empirical Formula**	**Monoisotopic Mass**	**log D (pH 5)**	**log D (pH 7)**		**Mean RT [min]**	**RSD [%]**	**Mean RT [min]**	**RSD [%]**	**RT [min]**	**RSD [%]**	**k**
Very polar	N,N-Bis(3-aminopropyl)methylamine	C7H19N3	145.1579	-10.06	-7.71	p	10.44	0.46	10.40	0.15	10.42	0.41	16.4
	Moroxydine	C6H13N5O	171.1120	-6.01	-5.43	p	11.46	0.53	11.29	0.17	11.37	0.86	18.0
	Cystine	C6H12N2O4S2	240.0238	-5.9	-5.9								
	Miglitol	C8H17NO5	207.1107	-5.74	-3.89	p	9.36	0.35	9.26	0.42	9.31	0.68	14.5
	Glyphosate	C3H8NO5P	169.0140	-5.12	-6.9								
	Diethanolamine	C4H11NO2	105.0790	-4.78	-3.79	p	8.03	1.00	7.81	0.36	7.92	1.58	12.2
	1-(N,N-Bis(2-hydroxyethyl)amino)propan-2-ol	C7H17NO3	163.1208	-4.76	-3.18	p	4.65	0.29	4.62	3.03	4.63	2.16	6.7
	Aminomethyl propanediol	C4H11NO2	105.0790	-4.68	-4.04	p	9.07	0.30	8.95	0.17	9.01	0.69	14.0
	2-(2-Aminoethoxy)ethanol	C4H11NO2	105.0790	-4.38	-3.71	p	7.47	1.09	7.31	0.36	7.39	1.36	11.3
	L-Asparagine	C4H8N2O3	132.0535	-4.29	-4.3								
	1H-Imidazole-1-propylamine	C6H11N3	125.0953	-4.26	-3.65	p	9.19	1.01	8.92	0.65	9.06	1.71	14.1
	1,3,4,5-Tetrahydroxycyclohexanecarboxylic acid	C7H12O6	192.0634	-4.24	-5.93								
	Acetylcholine	C7H16NO2	146.1176	-4.22	-4.22	p	7.59	0.67	7.45	0.69	7.52	1.17	11.5
	Histidine	C6H9N3O2	155.0695	-4.17	-3.7	p	18.07	0.19	18.23	0.62	18.15	0.63	29.3
	Acamprosate	C5H11NO4S	181.0409	-4.1	-4.1	p	11.86	0.28	11.81	0.23	11.83	0.32	18.7
	Phenformin	C10H15N5	205.1327	-4.1	-3.76	p	12.64	0.32	12.51	0.03	12.57	0.56	20.0
	Triisopropanolamine	C9H21NO3	191.1521	-4.06	-2.88	p	3.37	1.65	3.24	1.68	3.31	2.57	4.5
	Glutamic acid	C5H9NO4	147.0532	-4	-5.86								
	1,1'-Iminodipropan-2-Ol	C6H15NO2	133.1103	-3.96	-3.21	p	6.12	0.76	6.02	0.63	6.07	1.07	9.1
	2-[2-(Dimethylamino)ethoxy]ethanol	C6H15NO2	133.1103	-3.89	-2.41	p	4.22	2.87	3.86	0.42	4.04	4.97	5.7
	Piperidine	C2H6N4O	102.0542	-3.82	-2.36	n/p	8.91	0.49	8.85	0.35	8.88	0.54	13.8
	2-Ethylaminoethanol	C4H11NO	89.0841	-3.76	-3.26	p	6.59	0.93	6.45	0.33	6.52	1.27	9.9
	Aspartic acid	C4H7NO4	133.0375	-3.73	-5.37								

	Beatine	C5H11NO2	117.0790	-3.72	-3.72	p	8.98	1.38	8.56	0.94	8.77	2.70	13.6
	Tenofovir	C9H14N5O4P	287.0783	-3.71	-3.49								
	2-2'-Dimorpholinyldiethyl ether	C12H24N2O3	244.1787	-3.7	-0.71	p	3.19	2.11	2.81	1.79	3.00	6.73	4.0
	Metformin	C4H11N5	129.1014	-3.66	-3.66	p	11.61	0.61	11.41	0.18	11.51	0.97	18.2
	Aminomethylpropanol	C4H11NO	89.0841	-3.65	-3.35	p	7.36	0.45	7.23	0.39	7.30	0.99	11.2
	2-Pyrrolidin-1-ylethanol	C6H13NO	115.0997	-3.57	-2.69	p	6.10	1.12	5.84	0.86	5.97	2.41	8.9
	Threonine	C4H9NO3	119.0582	-3.47	-3.47								
	Famotidine	C8H15N7O2S3	337.0449	-3.43	-3.04	p	11.86	1.05	12.06	0.26	11.96	1.13	18.9
	Glycine	C2H5NO2	75.0320	-3.41	-3.18								
	3-Ethoxypropylamine	C5H13NO	103.0997	-3.29	-3.04	p	5.27	1.30	5.12	0.59	5.20	1.76	7.7
	2-(Dimethylamino)-2-methylpropan-1-ol	C6H15NO	117.1154	-3.27	-2.34	p	6.70	1.33	6.44	0.92	6.57	2.30	9.9
	Fructose	C6H12O6	180.0634	-3.27	-3.27	p	8.77	0.69	8.70	0.29	8.74	0.67	13.6
	Methylscopolamine	C18H24NO4	318.1700	-3.27	-3.27	p	15.09	0.82	14.62	0.31	14.86	1.70	23.8
	Trans-4-aminocyclohexan-1-ol	C6H13NO	115.0997	-3.24	-3	p	8.95	0.30	8.81	0.46	8.88	0.90	13.8
	beta-Alanine	C3H7NO2	89.0477	-3.21	-3.17								
	Clavulanate	C8H9NO5	199.0481	-3.21	-4.82								
	Sarcosine	C3H7NO2	89.0477	-3.19	-3.19	p	2.90	0.81	2.92	0.91	2.91	0.90	3.8
	Sotalol	C12H20N2O3S	272.1195	-3.18	-2.47	p	11.99	0.24	11.94	0.03	11.97	0.27	18.9
	Acetyllysine	C8H16N2O3	188.1161	-3.15	-3.15	p	11.81	0.17	11.83	0.77	11.82	0.56	18.7
	Lisinopril	C21H31N3O5	405.2264	-3.12	-3.13	p	7.37	0.38	7.22	0.43	7.30	1.09	11.2
	N-[3-(Dimethylamino)propyl]methacrylamide	C9H18N2O	170.1419	-3.01	-1.85	p	4.71	1.01	4.49	1.12	4.60	2.64	6.7
	Gamma-aminobutyric acid	C4H9NO2	103.0633	-2.99	-2.89	p	11.09	0.84	10.86	0.39	10.97	1.23	17.3
	Acesulfame	C4H5NO4S	162.9939	-2.97	-3.06	n/p	14.02	0.19	14.23	0.30	14.12	0.79	22.5
	Oxaceprol	C7H11NO4	173.0688	-2.96	-4.74	p	9.84	0.97	9.49	0.32	9.66	1.98	15.1
	Glucose	C6H12O6	180.0634	-2.93	-2.93	p	6.84	0.40	6.80	0.25	6.82	0.42	10.4
	2-Morpholinoethanol	C6H13NO2	131.0946	-2.89	-1.13	p	2.93	3.26	2.79	4.41	2.86	4.63	3.8
	Chlortetracycline	C22H23ClN2O8	478.1143	-2.89	-3.09								
	L-Alanine	C3H7NO2	89.0477	-2.84	-2.84	p	11.20	1.54	11.39	0.92	11.30	1.53	17.8
	Octopamine	C8H11NO2	153.0790	-2.83	-1.92	p	12.60	0.26	12.55	0.12	12.58	0.28	20.0

	Norfenefrine	C8H11NO2	153.0790	-2.83	-1.88	p	12.47	0.43	12.38	0.20	12.43	0.51	19.7
	Atenolol	C14H22N2O3	266.1630	-2.8	-2.14	p	10.67	0.63	10.50	0.26	10.59	0.93	16.6
	2-Butylaminoethanol	C6H15NO	117.1154	-2.79	-2.37	p	5.82	1.08	5.72	0.43	5.77	1.20	8.6
	Cefuroxime	C16H16N4O8S	424.0689	-2.75	-4.26								
	Diethylamine	C4H11N	73.0891	-2.72	-2.56	p	8.43	0.68	8.32	0.24	8.38	0.81	13.0
	2,2,6,6-Tetramethylpiperidin-4-ol	C9H19NO	157.1467	-2.72	-2.34	p	7.06	0.60	6.93	0.42	7.00	1.03	10.7
	2-6-Dimethylmorpholine	C6H13NO	115.0997	-2.7	-1.33	p	2.99	1.72	3.01	0.14	3.00	1.29	4.0
	Ethyl glycinate	C4H9NO2	103.0633	-2.69	-0.99	p	2.78	1.90	2.74	1.12	2.76	1.72	3.6
	Minoxidil	C9H15N5O	209.1277	-2.68	-2.25	p	7.06	0.35	7.05	1.53	7.06	1.11	10.8
	Dexrazoxane	C11H16N4O4	268.1172	-2.67	-2.65								
	Tetracycline	C22H24N2O8	444.1533	-2.65	-4.33								
	Taurine	C2H7NO3S	125.0147	-2.61	-2.62	p	14.03	0.25	13.95	0.26	13.99	0.39	22.3
	Butylamine	C10H21NO	171.1623	-2.59	-2.11	p	6.01	1.22	5.78	0.98	5.89	2.28	8.8
	Guanylurea	C5H11N	85.0891	-2.58	-2.36	p	5.86	0.91	5.72	0.38	5.79	1.40	8.6
	Pipamperone	C21H30FN3O2	375.2322	-2.57	0.13	p	5.45	0.84	5.29	0.84	5.37	1.68	7.9
	6-Amino-5-formamido-1-3-dimethyluracil	C3H6N6	126.0654	-2.54	-2.02	p	4.96	1.18	4.79	0.96	4.88	2.04	7.1
	Ectoine	C6H10N2O2	142.0742	-2.53	-2.53	p	10.10	1.25	9.73	0.61	9.92	2.12	15.5
	N,N-Dimethylisopropylamine	C5H13N	87.1048	-2.52	-1.9	p	3.71	2.09	3.46	2.07	3.59	3.97	5.0
Polar	4-Methylmorpholine	C5H11NO	101.0841	-2.5	-0.66	p	1.56	3.53	1.51	2.86	1.54	3.67	1.6
	Arecoline	C8H13NO2	155.0946	-2.39	-0.6	p	1.33	1.08	1.30	1.52	1.32	1.71	1.2
	Adenosine	C10H13N5O4	267.0968	-2.38	-2.1	p	6.40	0.67	6.31	0.81	6.36	1.02	9.6
	Allantoin	C4H6N4O3	158.0440	-2.36	-2.37	p	12.42	0.13	12.44	0.26	12.43	0.22	19.7
	L-Proline	C5H9NO2	115.0633	-2.34	-2.34	p	10.28	0.38	10.46	2.84	10.37	2.22	16.3
	1,2,2,6,6-Pentamethylpiperidin-4-ol	C4H11N	73.0891	-2.33	-2.11								
	Amoxicillin	C16H19N3O5S	365.1045	-2.31	-2.43								
	D(-) Ribose	C5H10O5	150.0528	-2.3	-2.3	p	6.65	0.10	6.62	0.54	6.64	0.47	10.1
	Etilefrine	C10H15NO2	181.1103	-2.26	-1.42	p	10.46	0.33	10.41	0.16	10.43	0.35	16.4
	Dopamine	C8H11NO2	153.0790	-2.24	-1.75	p	6.51	0.21	6.51	0.37	6.51	0.30	9.9
	Vigabatrin	C6H11NO2	129.0790	-2.21	-2.09	p	9.52	0.56	9.35	0.18	9.43	0.98	14.7

	L-Methionine	C5H11NO2S	149.0510	-2.19	-2.19	p	11.43	0.07	11.74	0.07	11.59	1.34	18.3
	Cefalexin	C16H17N3O4S	347.0940	-2.15	-2.26	p	9.90	0.24	10.04	0.22	9.97	0.74	15.6
	Isopentylamine	C5H13N	87.1048	-2.04	-1.82	p	4.83	0.73	4.65	0.86	4.74	2.07	6.9
	Maleic acid	C4H4O4	116.0110	-2.04	-4.55	n/p	12.27	0.40	12.36	0.39	12.31	0.54	19.5
	Dorzolamide	C10H16N2O4S3	324.0272	-2.04	-0.32	p	9.90	0.29	10.01	0.04	9.95	0.58	15.6
	Melamine	C7H10N4O3	198.0753	-2.02	-2.02	p	6.84	0.40	6.82	0.41	6.83	0.44	10.4
	4-Methylimidazole	C5H10N2	98.0844	-1.95	-0.3	p	1.11	5.37	1.11	3.97	1.11	4.72	0.8
	Valine	C5H11NO2	117.0790	-1.95	-1.95	p	2.78	0.68	2.75	1.19	2.77	1.10	3.6
	Tyramine	C8H11NO	137.0841	-1.94	-1.52	p	10.85	0.25	10.73	0.09	10.79	0.58	17.0
	Butylscopolamine	C21H30NO4	360.2169	-1.94	-1.94	p	13.23	0.57	12.93	0.18	13.08	1.21	20.8
	Diisopropylamine	C6H15N	101.1204	-1.89	-1.76	p	4.99	0.45	4.94	0.86	4.96	0.86	7.3
	2,2,6,6-Tetramethyl-4-piperidone	C9H17NO	155.1310	-1.87	-0.32	p	2.46	1.96	2.37	3.17	2.42	3.20	3.0
	[2-(Acryloyloxy)ethyl]trimethylammonium	C8H15NO2	157.1103	-1.83	-0.1	p	11.35	1.47	10.87	0.67	11.11	2.48	17.5
	1-(3'-Carboxypropyl)-3-7-dimethylxanthin	C11H14N4O4	266.1015	-1.8	-3.6	p	5.14	0.74	5.00	0.77	5.07	1.60	7.4
	N,N'-Ethylenedi(diacetamide)	C6H12N2O2	144.0899	-1.78	-1.78	p	3.66	1.30	3.62	3.43	3.64	2.65	5.1
	Panthenol	C9H19NO4	205.1314	-1.7	-1.7	p	5.21	0.38	5.18	0.91	5.19	0.75	7.7
	Cyanurodiamide (Atrazin-desethyl-desisopropyl-2-hydroxy)	C3H5N5O	127.0494	-1.69	-1.69	p	11.24	0.35	11.14	0.04	11.19	0.51	17.6
	Dacarbazine	C6H10N6O	182.0916	-1.69	-1.69	p	5.57	0.72	5.59	1.81	5.58	1.40	8.3
	Piracetam	C6H10N2O2	142.0742	-1.68	-1.68	p	3.87	1.36	3.83	0.60	3.85	1.18	5.4
	Dipropylamine	C6H15N	101.1204	-1.67	-1.56	p	4.17	1.42	4.11	0.30	4.14	1.30	5.9
	3-(2-Aminopropyl)phenol	C9H13NO	151.0997	-1.67	-1.19	p	9.74	0.26	9.67	0.15	9.70	0.45	15.2
	Oxypurinol	C5H4N4O2	152.0334	-1.67	-1.88	n/p	7.56	0.23	7.58	0.15	7.57	0.23	11.6
	Tranexamic acid	C8H15NO2	157.1103	-1.66	-1.55	p	10.07	0.60	9.89	0.33	9.98	0.99	15.6
	Ethylenediaminetetraacetonitrile	C10H12N6	216.1123	-1.65	-1.65	p	3.58	0.39	3.56	1.45	3.57	1.07	4.9
	Pyridoxine	C8H11NO3	169.0739	-1.58	-0.97	p	7.50	1.07	7.43	0.65	7.47	1.00	11.4
	(R)-1-(4-methoxyphenyl)ethylamine	C9H13NO	151.0997	-1.53	-1.19	p	8.82	0.38	8.67	0.20	8.74	0.89	13.6
	4-Hydroxybenzenesulfonic acid	C6H6O4S	173.9987	-1.53	-1.53	p	17.00	0.39	17.11	0.05	17.05	0.41	27.4
	D-α-methylbenzylamine	C8H11N	121.0891	-1.51	-1.04	p	4.90	0.52	4.74	0.82	4.82	1.79	7.0

	L-α-Methylbenzylamine	C8H11N	121.0891	-1.51	-1.04	p	4.89	0.27	4.77	0.78	4.83	1.35	7.1
	L-Isoleucine	C6H13NO2	131.0946	-1.51	-1.51								
	Pregabalin	C8H17NO2	159.1259	-1.51	-1.35	p	8.58	0.40	8.45	0.28	8.51	0.80	13.2
	Acetamide MEA	C4H9NO2	103.0633	-1.5	-1.5	p	3.76	0.54	3.64	1.15	3.70	1.87	5.2
	L-Tyrosine	C9H11NO3	181.0739	-1.49	-1.49	p	14.83	0.99	15.40	2.70	15.11	2.79	24.2
	Dimethylsulfoxide	C2H6OS	78.0139	-1.41	-1.41	p	1.21	1.38	1.18	2.58	1.19	2.50	1.0
	Gabapentin	C9H17NO2	171.1259	-1.4	-1.27	p	9.11	0.59	8.95	0.32	9.03	0.99	14.0
	N-(Hydroxymethyl)Acetamide	C3H7NO2	89.0477	-1.39	-1.39	p	4.04	1.07	3.93	1.87	3.98	2.06	5.6
	(2,4-Diaminopteridin-6-yl)yethanol	CH4N2O2	76.0273	-1.37	-1.37	p	5.85	0.47	5.95	1.11	5.90	1.20	8.8
	Hydroxycarbamide	C7H8N6O	192.0760	-1.37	-1.37	p	7.64	0.55	7.54	0.61	7.59	0.88	11.6
	Urea	CH4N2O	60.0324	-1.36	-1.36	p	7.83	0.26	7.79	0.41	7.81	0.44	12.0
	Tetraethylene glycol	C8H18O5	194.1154	-1.35	-1.35	p	3.31	0.38	3.10	1.14	3.20	3.46	4.3
	Ferulic acid	C14H21NO4	267.1471	-1.25	-1.24	p	13.58	0.65	13.30	0.25	13.44	1.14	21.4
	Cimetidine	C10H16N6S	252.1157	-1.24	-0.34	n/p	8.53	0.82	8.42	0.50	8.48	0.94	13.1
	Tolazoline	C10H12N2	160.1000	-1.21	-1.15	p	9.16	0.97	8.93	0.44	9.05	1.51	14.1
	Phenylalanine	C9H11NO2	165.0790	-1.19	-1.19	p	11.02	1.42	11.48	0.12	11.25	2.26	17.7
	4-Methylmorpholine 4-oxide	C5H11NO2	117.0790	-1.16	-1.15	p	4.37	1.33	4.14	2.57	4.26	3.41	6.1
	Cytosine	C4H5N3O	111.0433	-1.15	-1.15	p	8.43	0.68	8.32	0.24	8.38	0.81	13.0
	Cyanuric acid	C2H6N2O	74.0480	-1.14	-1.14	p	4.66	0.45	4.65	0.98	4.65	0.77	6.8
	Tryptophan	C11H12N2O2	204.0899	-1.09	-1.09								
	2-Methylimidazole	C4H6N2	82.0531	-1.08	-0.55	p	4.91	0.96	4.80	0.96	4.85	1.49	7.1
	Adenine	C5H5N5	135.0545	-1.05	-0.58	p	4.28	0.95	4.25	1.22	4.27	1.14	6.1
	14-Hydroxyclarithromycin	C38H69NO14	763.4718	-1.05	0.69	p	6.58	0.93	6.37	0.67	6.48	1.79	9.8
	Methylurea	C3H3N3O3	129.0174	-1.04	-1.14								
	Cyanoguanidine	C2H4N4	84.0436	-1.03	-1.03	n/p	8.17	0.13	8.19	0.20	8.18	0.20	12.6
	Perhydropyrimidin-2-one	C4H8N2O	100.0637	-1.03	-1.03	p	3.37	0.43	3.23	1.13	3.30	2.34	4.5
	Propylidynetrimethanol	C6H14O3	134.0943	-1.01	-1.01								
	Riboflavin	C17H20N4O6	376.1383	-0.92	-1.23	p	14.32	0.48	14.08	0.08	14.20	0.94	22.7
	Emtricitabine	C8H10FN3O3S	247.0427	-0.9	-0.9	p	6.88	0.39	6.84	0.76	6.86	0.65	10.4

	1,2-Dimethylimidazole	C5H8N2	96.0687	-0.85	-0.25	p	3.30	0.56	3.38	2.03	3.34	1.93	4.6
	N-Formylmorpholine	C5H9NO2	115.0633	-0.85	-0.85	p	1.09	3.14	1.04	2.60	1.06	3.49	0.8
	2-Hydroxy-N,N-dimethyl-propanamide	C5H11NO2	117.0790	-0.83	-0.83	p	1.11	2.48	1.09	1.97	1.10	2.33	0.8
	N-(Hydroxymethyl)nicotinamide	C7H8N2O2	152.0586	-0.77	-0.75	p	4.51	0.61	4.46	1.17	4.48	1.07	6.5
	N-(2-Hydroxyethyl)prop-2-enamide	C5H9NO2	115.0633	-0.74	-0.74	n/p	3.69	1.43	3.67	2.08	3.68	1.79	5.1
	1,4-Butinodiol	C4H6O2	86.0368	-0.73	-0.73	p	2.78	1.90	2.74	1.12	2.76	1.72	3.6
	Lenalidomide	C13H13N3O3	259.0957	-0.71	-0.71	p	11.38	0.62	11.18	0.43	11.28	1.02	17.8
	Isoniazid	C6H7N3O	137.0589	-0.7	-0.69	p	4.63	0.69	4.47	1.75	4.55	2.14	6.6
	Methoxytriglycol	C7H16O4	164.1049	-0.66	-0.66	p	1.15	1.38	1.11	2.81	1.13	2.59	0.9
	1,3-Dimethylimidazolidin-2-one	C5H10N2O	114.0793	-0.64	-0.64	p	1.06	0.81	1.02	2.73	1.04	2.74	0.7
	2-Ethylhexylamine	C8H19N	129.1517	-0.63	-0.42	p	4.05	1.06	3.94	1.10	3.99	1.76	5.7
	Venlafaxine	C17H27NO2	277.2042	-0.62	0.84	p	3.56	0.85	3.40	1.91	3.48	2.69	4.8
	2-Pyrrolidone	C4H7NO	85.0528	-0.58	-0.58	p	3.68	0.71	3.62	3.43	3.65	2.58	5.1
	Hydrochlorthiazide	C7H8ClN3O4S2	296.9645	-0.58	-0.58	n/p	17.26	0.32	17.61	0.38	17.44	1.09	28.1
	Omethoate	C5H12NO4PS	213.0225	-0.55	-0.55	p	2.60	3.71	2.88	2.32	2.74	5.95	3.6
	Asulam	C8H10N2O4S	230.0361	-0.55	-0.58	p	10.23	0.10	10.29	0.04	10.26	0.30	16.1
	3-Dimethylaminopropiononitrile	C4H6N2	82.0531	-0.53	-0.3	p	4.34	0.75	4.30	0.28	4.32	0.68	6.2
	1-Methylimidazole	C4H6N2	82.0531	-0.49	-0.07	p	1.96	1.50	1.98	0.75	1.97	1.30	2.3
	Sucralose	C12H19Cl3O8	396.0146	-0.47	-0.47	p	12.62	0.10	12.73	0.14	12.68	0.46	20.1
	Iopromide	C18H24I3N3O8	790.8698	-0.44	-0.44	p	13.81	0.33	13.76	0.29	13.79	0.35	22.0
	Ethyl 2-oxopyrrolidine-1-acetate	C8H13NO3	171.0895	-0.38	-0.38	p	1.10	0.22	1.11	0.99	1.10	0.85	0.8
	Ritalinic Acid	C13H17NO2	219.1259	-0.38	-0.36	p	9.09	0.74	8.86	0.37	8.97	1.38	14.0
	Diethylene glycol ethyl ether	C6H14O3	134.0943	-0.26	-0.26	p	1.04	1.55	1.03	1.64	1.03	1.66	0.7
	Methyl 3-aminocrotonate	C5H9NO2	115.0633	-0.24	-0.24	p	3.80	0.73	3.74	0.75	3.77	1.16	5.3
	Nicotinylalcohol	C6H7NO	109.0528	-0.17	-0.01	p	2.36	5.75	2.39	0.79	2.37	4.13	3.0
	6-Mercaptopurine	C5H4N4S	152.0157	-0.13	-0.12	p	8.03	0.62	8.07	0.39	8.05	0.56	12.4
	2-Mercaptoethanol	C2H6OS	78.0139	-0.11	-0.11	p	1.31	0.62	1.23	4.46	1.27	4.51	1.1
	Ipral	C14H18N4O3	290.1379	-0.11	0.92	p	6.90	0.62	6.79	0.38	6.84	0.93	10.4
	1,4-Dioxane	C4H8O2	88.0524	-0.09	-0.09	p	8.03	1.00	7.81	0.36	7.92	1.58	12.2

	Syringic acid	C9H10O5	198.0528	-0.09	-1.93	p	7.27	0.55	7.42	2.31	7.35	1.98	11.2
	Tetraglyme	C10H22O5	222.1467	-0.06	-0.06	p	1.00	0.97	1.02	2.33	1.01	2.07	0.7
	2-Phenyl-1H-benzimidazole-5-sulphonic acid	C13H10N2O3S	274.0412	0	0.09	p	18.37	0.52	18.36	0.49	18.37	0.51	29.6
	Diatrizoate	C11H9I3N2O4	613.7697	0.14	-0.62	p	16.59	0.30	16.45	0.08	16.52	0.47	26.5
	Phenylpyruvic acid	C9H8O3	164.0473	0.23	-1.39								
	Oxadixyl	C17H21NO	255.1623	0.31	1.79	p	3.02	1.90	2.92	1.01	2.97	2.28	4.0
	Cygon (Dimethoate)	C5H12NO3PS2	228.9996	0.34	0.34	p	1.94	1.47	1.92	2.28	1.93	1.97	2.2
	Methacrylic Acid	C4H6O2	86.0368	0.55	-1.21	p	11.09	0.84	10.84	0.54	10.96	1.34	17.3
	Vasotec	C20H28N2O5	376.1998	0.55	-0.85	p	6.20	0.88	5.98	0.65	6.09	1.97	9.1
	Atenolol acid (metoprolol acid)	C10H10O4	194.0579	0.61	-1.24	p	7.24	0.45	7.24	0.66	7.24	0.57	11.1
	Norfluoxetine	C16H16F3NO	295.1184	0.72	1.16	p	6.13	0.54	6.10	0.72	6.11	0.68	9.2
	Sulfamethoxazole	C10H11N3O3S	253.0521	0.76	0.14	p	9.02	0.01	9.10	0.04	9.06	0.46	14.1
	2-5-Dimethoxyaniline	C8H11NO2	153.0790	0.8	0.83	p	1.16	1.88	1.16	0.32	1.16	1.35	0.9
	Acetaminophen	C8H9NO2	151.0633	0.91	0.91	n/p	7.03	0.24	7.05	0.33	7.04	0.31	10.7
	Fluoxetine	C17H18F3NO	309.1340	0.94	1.5	p	4.63	1.32	4.49	0.79	4.56	1.97	6.6
	P-Coumaric acid	C9H8O3	164.0473	0.97	-0.89	n/p	8.03	0.61	8.03	0.69	8.03	0.65	12.4
	P-Phenetidine	C8H11NO	137.0841	0.98	1.34	p	1.39	0.80	1.38	2.23	1.38	1.72	1.3
	Chloridazon	C10H8ClN3O	221.0356	1.11	1.11	n/p	6.18	0.52	6.18	0.59	6.18	0.55	9.3
	Primidone	C12H14N2O2	218.1055	1.12	1.12	p	4.91	0.44	4.89	0.82	4.90	0.68	7.2
	Triethylphosphate	C6H15O4P	182.0708	1.18	1.18	p	0.76	1.99	0.77	2.51	0.76	2.42	0.3
	O-Phenetidine	C8H11NO	137.0841	1.24	1.34	p	1.04	2.31	1.01	2.75	1.03	3.03	0.7
	Dapsone	C12H12N2O2S	248.0619	1.27	1.27	p	11.48	0.22	11.49	0.19	11.48	0.22	18.1
	Acifluorfen	C14H7ClF3NO5	360.9965	1.27	1.03	p	8.97	0.07	8.99	0.10	8.98	0.17	14.0
	Amitriptyline	C20H23N	277.1830	1.33	2.12	p	3.24	3.44	3.07	2.35	3.15	3.97	4.3
	6-Methoxy-m-toluidine	C8H11NO	137.0841	1.35	1.5	p	1.04	2.10	0.97	1.01	1.01	3.73	0.7
	1-2-Benzisothiazol-3(2H)-one	C7H5NOS	151.0092	1.36	1.36	p	2.22	1.35	2.31	1.79	2.27	2.58	2.8
	Carboxin	C12H13NO2S	235.0667	1.51	1.51	p	1.70	1.68	1.69	0.89	1.69	1.36	1.8
	Azamethiphos	C9H10ClN2O5PS	323.9737	1.52	1.52	p	1.17	1.35	1.14	2.16	1.15	2.32	0.9
	Hexahydro-4-methylphthalic anhydride	C9H12O3	168.0786	1.53	1.53	p	7.80	0.56	7.85	0.20	7.83	0.52	12.0

	Hexanoic acid	C6H12O2	116.0837	1.55	-0.09								
	Bayrepel	C12H23NO3	229.1678	1.61	1.61	p	0.97	1.65	0.98	1.38	0.98	1.67	0.6
	Carbetamide	C12H16N2O3	236.1161	1.65	1.65	p	2.78	0.68	2.77	0.86	2.77	0.85	3.6
	Methylparaben	C8H8O3	152.0473	1.67	1.66	p	2.34	2.95	2.36	2.80	2.35	2.90	2.9
	Chlorsulfuron	C12H12ClN5O4S	357.0299	1.67	1.56	p	8.08	0.35	8.10	0.28	8.09	0.33	12.5
	Hymecromone	C10H8O3	176.0473	1.78	1.72	p	3.43	1.13	3.43	2.30	3.43	1.82	4.7
	(+)-Catechin	C14H18N2O4	278.1267	1.79	1.79	p	2.24	2.12	2.33	6.16	2.28	5.07	2.8
	Diphenhydramine	C15H14O6	290.0790	1.8	1.79	p	6.41	1.04	6.30	0.61	6.36	1.22	9.6
	Malathion	C10H19O6PS2	330.0361	1.86	1.86	p	0.96	1.64	0.97	0.82	0.96	1.33	0.6
	Monuron	C9H11ClN2O	198.0560	1.93	1.93								
	Metribuzin	C8H14N4OS	214.0888	1.96	1.96	p	1.29	2.24	1.28	2.55	1.29	2.43	1.1
Non-polar	Haloxyfop	C15H11ClF3NO4	361.0329	2.02	0.77	p	5.88	0.66	5.77	0.54	5.82	1.08	8.7
	2,6-Dichlorbenzamide	C7H5Cl2NO	188.9748	2.03	2.03	p	3.68	0.59	3.64	1.24	3.66	1.08	5.1
	Carbofuran	C12H15NO3	221.1052	2.05	2.05	p	1.31	0.60	1.27	0.99	1.29	1.56	1.2
	Naproxen	C14H14O3	230.0943	2.11	0.25	p	3.12	0.73	3.09	0.34	3.11	0.76	4.2
	TCEP	C6H12Cl3O4P	283.9539	2.11	2.11	p	1.13	1.41	1.12	1.05	1.13	1.41	0.9
	Dilantin	C15H12N2O2	252.0899	2.15	2.15	p	4.14	0.68	4.19	0.88	4.17	0.99	5.9
	Atrazine	C8H14ClN5	215.0938	2.19	2.2	p	1.03	1.39	1.02	5.38	1.03	3.95	0.7
	Metobromuron	C9H11BrN2O2	258.0004	2.24	2.24	p	1.28	1.27	1.28	2.30	1.28	1.86	1.1
	Indomethacin	C19H16ClNO4	357.0768	2.31	0.5	p	4.59	1.13	4.54	0.98	4.57	1.22	6.6
	Molinate	C9H17NOS	187.1031	2.34	2.34	p	0.93	3.44	0.90	3.11	0.92	3.88	0.5
	Linuron	C9H13N	135.1048	2.4	2.68	p	1.04	1.33	1.03	2.44	1.03	2.08	0.7
	Candesartan	C24H20N6O3	440.1597	2.4	-0.12	p	15.60	0.54	15.39	0.06	15.50	0.79	24.8
	Chlorotoluron	C10H13ClN2O	212.0716	2.44	2.44	n/p	2.21	1.52	2.21	2.39	2.21	2.00	2.7
	N,N-Diethyl-meta-toluamide	C12H17NO	191.1310	2.5	2.5	p	1.00	3.24	0.98	2.61	0.99	3.18	0.7
	Propylparaben	C10H12O3	180.0786	2.55	2.54	p	2.10	1.47	2.22	1.42	2.16	3.16	2.6
	Dimethyl sebacate	C12H22O4	230.1518	2.56	2.56	p	0.69	0.89	0.67	1.85	0.68	1.97	0.1
	2-Ethyl-N-(2-ethylhexyl)-1-hexanamine	C16H35N	241.2770	2.61	2.67	p	1.25	3.36	1.22	2.87	1.24	3.34	1.1
	Estriol	C18H24O3	288.1725	2.67	2.67	p	7.86	0.08	7.90	0.16	7.88	0.27	12.1

	Pseudocumidine	C9H10Cl2N2O2	248.0119	2.68	2.68	n/p	1.39	1.97	1.47	9.05	1.43	7.23	1.4
	Budesonide	C25H34O6	430.2355	2.73	2.73	n/p	4.46	0.54	4.45	0.96	4.45	0.79	6.4
	Carbamazepine	C15H12N2O	236.0950	2.77	2.77	p	2.79	1.41	2.72	0.98	2.76	1.80	3.6
	Chlorbromuron	C9H10BrClN2O2	291.9614	2.85	2.85	n/p	1.37	2.94	1.37	3.04	1.37	2.99	1.3
	Diphenamide	C16H17NO	239.1310	2.93	2.93	p	1.27	1.29	1.27	2.32	1.27	1.88	1.1
	Diclofenac	C9H18O2	158.1307	2.94	1.37	n/p	6.40	1.25	6.15	1.53	6.28	2.41	9.5
	Metazachlor	C14H16ClN3O	277.0982	2.98	2.98	p	1.05	1.61	1.09	2.60	1.07	2.84	0.8
	Diethofencarb	C14H21NO4	267.1471	3	3	p	1.12	2.53	1.09	5.33	1.11	4.34	0.8
	Butylated Hydroxyanisole	C11H16O2	180.1150	3.06	3.06	p	8.16	1.44	7.98	0.33	8.07	1.54	12.4
	Chlorpropham	C10H12ClNO2	213.0557	3.21	3.21	p	1.19	0.14	1.17	2.38	1.18	1.84	1.0
	Pelargonic acid	C14H11Cl2NO2	295.0167	3.21	1.37	n/p	6.40	1.25	6.15	1.53	6.28	2.41	9.5
	Flufenacet	C14H13F4N3O2S	363.0665	3.22	3.22	p	0.89	0.82	0.88	1.11	0.89	1.04	0.5
	1-4-Dioxacyclohexadecane-5-16-dione	C14H24O4	256.1675	3.28	3.28	p	3.02	1.90	2.92	1.01	2.97	2.28	4.0
	Ethyl [2-(4-phenoxyphenoxy)ethyl]carbamate (Fenoxycarb)	C17H19NO4	301.1314	3.31	3.31	p	1.09	2.27	1.10	1.13	1.09	1.80	0.8
	TCPP	C9H18Cl3O4P	326.0008	3.36	3.36	p	0.91	1.53	0.91	4.10	0.91	3.09	0.5
	Testosterone	C19H28O2	288.2089	3.37	3.37	p	1.82	1.12	1.80	0.76	1.81	1.11	2.0
	Benzophenone	C13H10O	182.0732	3.43	3.43	p	1.03	0.79	1.01	2.10	1.02	1.80	0.7
	Metolachlor	C15H22ClNO2	283.1339	3.45	3.45	p	0.96	1.64	0.99	2.54	0.98	2.73	0.6
	Ibuprofen	C13H18O2	206.1307	3.46	1.71	p	1.95	0.99	1.93	0.11	1.94	0.90	2.2
	Alachlor	C14H20ClNO2	269.1183	3.59	3.59	p	0.90	2.39	0.91	1.34	0.91	2.10	0.5
	Metconazole	C17H22ClN3O	319.1451	3.59	3.59	p	1.78	1.76	1.73	2.81	1.76	2.72	1.9
	Benzophenone-3 (Oxybenzone)	C14H12O3	228.0786	3.62	3.36	p	0.85	0.86	0.97	10.74	0.91	10.52	0.5
	Spironolacton	C24H32O4S	416.2021	3.64	3.64	p	1.82	0.86	1.80	0.94	1.81	1.06	2.0
	17-Beta-Estradiol	C18H24O2	272.1776	3.75	3.75	p	5.54	0.50	5.55	0.34	5.55	0.43	8.2
	Acetyl Cedrene	C17H26O	246.1984	3.87	3.87	p	0.86	0.43	0.86	2.57	0.86	1.85	0.4
	Ethinylestradiol	C20H24O2	296.1776	3.9	3.9	p	5.86	0.16	5.90	0.31	5.88	0.43	8.8
	Bisphenol A	C15H16O2	228.1150	4.04	4.04	n/p	7.06	0.25	7.16	0.35	7.11	0.73	10.8
	Diazinon	C12H21N2O3PS	304.1011	4.13	4.19	p	0.73	2.83	0.81	10.56	0.77	9.83	0.3

	Prosulfocarb	C14H21NOS	251.1344	4.17	4.17	p	0.77	0.95	0.79	3.75	0.78	3.16	0.3
	TDCPP	C9H15Cl6O4P	427.8839	4.28	4.28	p	1.12	0.70	1.10	2.44	1.11	2.00	0.8
	Chlorfenvinphos	C12H14Cl3O4P	357.9695	4.3	4.3	p	0.91	3.25	0.90	1.95	0.91	2.71	0.5
	Estrone	C18H22O2	270.1620	4.31	4.31	p	2.73	0.50	2.76	0.88	2.74	0.91	3.6
	Picoxystrobin	C18H16F3NO4	367.1031	4.31	4.31	p	1.04	0.41	1.02	3.26	1.03	2.66	0.7
	Valsartan	C24H29N5O3	435.2270	4.54	2.54	p	12.39	0.39	12.27	0.03	12.33	0.57	19.6
	Flurtamone	C18H14F3NO2	333.0977	4.64	4.64	p	3.28	1.66	3.20	2.13	3.24	2.26	4.4
	Pyraclostrobin	C19H18ClN3O4	387.0986	4.7	4.7	p	1.30	1.07	1.30	2.86	1.30	2.16	1.2
	Profenofos	C11H15BrClO3PS	371.9351	4.88	4.88	p	0.91	1.53	0.82	13.69	0.87	10.63	0.4
	Boscalid	C18H12Cl2N2O	342.0327	4.92	4.92	n/p	2.20	8.33	2.07	3.33	2.14	7.19	2.6
	Triclocarban	C13H9Cl3N2O	313.9780	4.93	4.93	p	7.09	2.39	7.36	0.52	7.22	2.50	11.0
	Quinoxyfen	C15H8Cl2FNO	306.9967	4.95	4.98	p	0.93	3.88	0.91	4.75	0.92	4.40	0.5
	Triclosan	C12H7Cl3O2	287.9512	4.98	4.9	p	1.12	0.70	1.12	0.58	1.12	0.65	0.9
	Myristic acid	C14H28O2	228.2089	5.04	3.33	p	1.39	1.33	1.38	2.04	1.39	1.76	1.3
	Fenofibrate	C20H21ClO4	360.1128	5.28	5.28	p	0.93	3.73	0.91	4.43	0.92	4.20	0.5
	Fenazaquin	C20H22N2O	306.1732	5.42	5.42	p	0.93	3.73	0.91	4.43	0.92	4.20	0.5
	4-(7-Methyloctyl)phenol	C15H24O	220.1827	5.58	5.58								
	Linoleic acid	C18H32O2	280.2402	6.12	4.42	p	1.43	1.31	1.43	2.43	1.43	1.97	1.4
	Dibenzothiazyl disulfide	C14H8N2S4	331.9570	6.22	6.22								
	Stearic acid	C18H36O2	284.2715	6.82	5.11								
	6-6'-Di-tert-butyl-4-4'-thiodi-m-cresol	C22H30O2S	358.1967	7.67	7.67								

Table S- 13: RTs of standard compounds and RT and RSDs of compounds which were detected in the wastewater treatment plant effluent sample by RPLC-HILIC/TOF-MS and/or SFC/TOF-MS. Deviations of RTs were calculated by differences of standard compound RT and those from the environmental water sample.

						RPLC-HILIC/TOF-MS					SFC/TOF-MS			
	Compound name	**Empirical Formula**	**Monoisotopic Mass**	**log D (pH 5)**	**log D (pH 7)**	**RT standards [min]**	**RT water sample [min]**	**RSD water sample [%]**	**Deviation of RTs [%]**		**RT standards [min]**	**RT water sample [min]**	**SD water sample [min]**	**Deviation of RTs [%]**
Very polar	Maleic acid	$C_4H_4O_4$	116.0110	-2.04	-4.55	5.30	5.49	0.52	3.53		12.31			
	Betaine	$C_5H_{11}NO_2$	117.0790	-3.72	-3.72	11.71	11.77	0.27	0.54		8.77	9.19	0.11	4.78
	Metformin	$C_4H_{11}N_5$	129.1014	-3.66	-3.66	15.98	15.98	1.56	0.03		11.51	11.36	0.06	0.49
	Tenofovir	$C_9H_{14}N_5O_4P$	287.0783	-3.71	-3.49	11.99	11.93	0.35	0.47					
	Threonine	$C_4H_9NO_3$	119.0582	-3.47	-3.47	13.07	13.25	0.01	1.36					
	Glycine	$C_2H_5NO_2$	75.0320	-3.41	-3.41	13.35	13.46	0.16	0.80					
	1,1'-Iminodipropan-2-ol	$C_6H_{15}NO_2$	133.1103	-3.96	-3.21	15.27	15.39	1.17	0.79		6.07			
	beta-Alanine	$C_3H_7NO_2$	89.0477	-3.21	-3.17	13.47	13.53	0.06	0.42					
	3-Ethoxypropylamine	$C_5H_{13}NO$	103.0997	-3.29	-3.04	11.86	11.68	0.07	1.56		5.20			
	Trans-4-aminocyclohexan-1-ol	$C_6H_{13}NO$	115.0997	-3.24	-3	20.77					8.88	8.76	0.03	1.35
	Gamma-aminobutyric acid	$C_4H_9NO_2$	103.0633	-2.99	-2.89	13.68	13.73	0.06	0.32		10.97			
	Triisopropanolamine	$C_9H_{21}NO_3$	191.1521	-4.06	-2.88	6.95	6.89	0.42	0.86		3.31			
	L-Alanine	$C_3H_7NO_2$	89.0477	-2.84	-2.84	12.97	13.03	0.18	0.46		11.30			
	Taurine	$C_2H_7NO_3S$	125.0147	-2.61	-2.62	12.64	12.70	0.17	0.52		13.99			
Polar	2-Butylaminoethanol	$C_6H_{15}NO$	117.1154	-2.79	-2.37	11.63					5.77	5.94	0.00	2.84
	Piperidine	$C_5H_{11}N$	85.0891	-2.58	-2.36	21.43					5.79	5.87	0.04	1.46
	Guanylurea	$C_2H_6N_4O$	102.0542	-3.82	-2.36	14.61	14.84	1.02	1.58		8.88	8.89	0.04	0.13
	L-Proline	$C_5H_9NO_2$	115.0633	-2.34	-2.34	12.21	12.37	0.29	1.27		10.37			
	2-(Dimethylamino)-2-methylpropan-1-ol	$C_6H_{15}NO$	117.1154	-3.27	-2.34	19.31					6.57	6.60	0.04	0.53
	2,2,6,6-Tetramethylpiperidin-4-ol	$C_9H_{19}NO$	157.1467	-2.72	-2.34						7.00	7.04	0.05	0.62
	1,2,2,6,6-Pentamethylpiperidin-4-ol	$C_{10}H_{21}NO$	171.1623	-2.59	-2.11						5.89	6.04	0.07	2.48

	Adenosine	C10H13N5O4	267.0968	-2.38	-2.1	7.31	7.44	0.29	1.74		6.36	6.50	0.02	2.23
	Melamine	C3H6N6	126.0654	-2.54	-2.02	7.44	7.53	0.19	1.15		4.88	5.04	0.03	3.33
	6-Amino-5-formamido-1-3-dimethyluracil	C7H10N4O3	198.0753	-2.02	-2.02	6.77	6.50	0.56	4.04		6.83			
	Valine	C5H11NO2	117.0790	-1.95	-1.95	11.74					2.77	2.86	0.03	3.38
	N-[3-(Dimethylamino)propyl]methacrylamide	C9H18N2O	170.1419	-3.01	-1.85	25.18	25.12	0.09	0.26		4.60			
	Isopentylamine	C5H13N	87.1048	-2.04	-1.82	10.92					4.74	4.94	0.04	4.08
	N,N'-Ethylenedi(diacetamide)	C6H12N2O2	144.0899	-1.78	-1.78	6.25	6.41	0.14	2.60		3.64	3.59	0.01	1.43
	Diisopropylamine	C6H15N	101.1204	-1.89	-1.76	11.06	11.11	0.25	0.48		4.96	4.83	0.03	2.72
	Panthenol	C9H19NO4	205.1314	-1.7	-1.7	6.60	6.39	0.34	3.25		5.19			
	Piracetam	C6H10N2O2	142.0742	-1.68	-1.68	6.29	6.52	0.12	3.66		3.85			
	Dipropylamine	C6H15N	101.1204	-1.67	-1.56	10.89					4.14	4.20	0.02	1.34
	L-Isoleucine	C6H13NO2	131.0946	-1.51	-1.51	10.97	10.86	0.13	0.93					
	Acetamide MEA	C4H9NO2	103.0633	-1.5	-1.5	6.17	6.27	0.55	1.59		3.70	3.67	0.04	1.02
	Dimethylsulfoxide	C2H6OS	78.0139	-1.41	-1.41	5.95	6.06	0.01	1.85		1.19			
	N-(Hydroxymethyl)acetamide	C3H7NO2	89.0477	-1.39	-1.39	12.87	13.03	0.18	1.22		3.98	3.99	0.01	0.14
	Pregabalin	C8H17NO2	159.1259	-1.51	-1.35	10.10	10.16	0.21	0.60		8.51	8.59	0.04	0.89
	Tetraethylene glycol	C8H18O5	194.1154	-1.35	-1.35	6.44	6.36	0.23	1.27		3.20	3.11	0.03	2.80
	Gabapentin	C9H17NO2	171.1259	-1.4	-1.27	10.08	9.91	0.33	1.75		9.03	9.12	0.05	1.02
	Atenolol acid (metoprolol acid)	C14H21NO4	267.1471	-1.25	-1.24	12.49	12.51	0.35	0.13		13.44	13.69	0.06	1.86
	Methacrylic Acid	C4H6O2	86.0368	0.55	-1.21	13.68	13.71	0.01	0.23		10.96			
	3-(2-Aminopropyl)phenol	C9H13NO	151.0997	-1.53	-1.19	11.37	11.38	0.42	0.07		8.74			
	Cytosine	C4H5N3O	111.0433	-1.15	-1.15	8.12	7.86	0.37	3.22		8.38	8.57	0.06	2.29
	4-Methylmorpholine 4-oxide	C5H11NO2	117.0790	-1.16	-1.15	12.44	12.53	0.06	0.70		4.26			
	Methylurea	C2H6N2O	74.0480	-1.14	-1.14	6.17	6.29	0.14	1.90		4.65	4.71	0.01	1.29
	2-Morpholinoethanol	C6H13NO2	131.0946	-2.89	-1.13	6.95	7.16	0.30	2.94		2.86			
	Cyanoguanidine	C2H4N4	84.0436	-1.03	-1.03						8.18	8.16	0.01	0.23
	Pyridoxine	C8H11NO3	169.0739	-1.58	-0.97	6.77	6.77	1.42	0.06		7.47			

	N-(Hydroxymethyl)nicotinamide	C7H8N2O2	152.0586	-0.77	-0.75	6.52					4.48	4.56	0.01	1.57
	1,4-Butinodiol	C4H6O2	86.0368	-0.73	-0.73	6.42	6.25	2.73	2.76		2.76			
	Diatrizoate	C11H9I3N2O4	613.7697	0.14	-0.62	9.10					16.52	16.70	0.05	1.07
	2-Pyrrolidone	C4H7NO	85.0528	-0.58	-0.58	6.28	6.41	0.33	2.05		3.65			
	Adenine	C5H5N5	135.0545	-1.05	-0.58	7.01	7.20	0.30	2.80		4.27	4.45	0.01	4.29
	2-Methylimidazole	C4H6N2	82.0531	-1.08	-0.55	7.43					4.85	4.97	0.04	2.45
	Sucralose	C12H19Cl3O8	396.0146	-0.47	-0.47	22.86	22.87	0.30	0.06		12.68			
	Iopromide	C18H24I3N3O8	790.8698	-0.44	-0.44	8.63	8.46	0.44	1.95		13.79			
	2-Ethylhexylamine	C8H19N	129.1517	-0.63	-0.42	27.68					3.99	3.95	0.02	0.63
	Ritalinic Acid	C13H17NO2	219.1259	-0.38	-0.36	8.38	8.51	0.83	1.48		8.97	9.10	0.05	1.44
	2,2,6,6-Tetramethyl-4-piperidone	C9H17NO	155.1310	-1.87	-0.32	6.55	6.47	0.13	1.26		2.42			
	4-Methylimidazole	C4H6N2	82.0531	-0.53	-0.3	6.88	6.89	0.24	0.11		4.32	4.44	0.02	2.69
	1,2-Dimethylimidazole	C5H8N2	96.0687	-0.85	-0.25	7.69	7.38	0.38	3.98		3.34			
	Methyl 3-aminocrotonate	C5H9NO2	115.0633	-0.24	-0.24	6.35	6.60	0.01	3.92		3.77			
	Candesartan	C24H20N6O3	440.1597	2.4	-0.12	23.26					15.50	15.70	0.06	1.33
	2-Mercaptoethanol	C2H6OS	78.0139	-0.11	-0.11	5.96	6.06	0.01	1.76		1.27	1.31	0.04	3.09
	[2-(Acryloyloxy)ethyl]trimethylammonium	C8H15NO2	157.1103	-1.83	-0.1	10.87	10.81	0.22	0.56		11.11			
	1,4-Dioxane	C4H8O2	88.0524	-0.09	-0.09	19.92					7.92	8.05	0.05	1.70
	Sulfamethoxazole	C10H11N3O3S	253.0521	0.76	0.14	17.50					9.06	9.01	0.02	0.54
	14-Hydroxyclarithromycin	C38H69NO14	763.4718	-1.05	0.69	27.32					6.48	6.62	0.05	2.30
	2-5-Dimethoxyaniline	C8H11NO2	153.0790	0.8	0.83	25.36	25.22	0.10	0.56		1.16			
	Venlafaxine	C17H27NO2	277.2042	-0.62	0.84	27.61	27.45	0.13	0.57		3.48			
	Ipral	C14H18N4O3	290.1379	-0.11	0.92	24.15	24.11	0.09	0.17		6.84			
	Pelargonic acid	C9H18O2	158.1307	2.94	1.37	26.31	26.36	0.10	0.19					
	Diclofenac	C14H11Cl2NO2	295.0167	3.21	1.37	25.67	25.65	0.08	0.07		6.28	6.14	0.14	2.18
Non-polar	TCEP	C6H12Cl3O4P	283.9539	2.11	2.11	27.12					1.13	1.11	0.03	1.92
	N,N-Diethyl-meta-toluamide	C12H17NO	191.1310	2.5	2.5	27.69	27.67	0.02	0.07		0.99			

	Valsartan	C24H29N5O3	435.2270	4.54	2.54	24.05	24.06	0.08	0.06		12.33	12.43	0.03	0.84
	Carbamazepine	C15H12N2O	236.0950	2.77	2.77	26.41	26.42	0.03	0.04		2.76			
	TDCPP	C9H15Cl6O4P	427.8839	4.28	4.28	31.05					1.11	1.08	0.00	2.71
	Triclosan	C12H7Cl3O2	287.9512	4.98	4.9	32.53					1.12	1.11	0.03	1.22

Table S- 14: MS signal areas of compounds analyzed as mix in ACN/water (50/50, v/v) (standard conditions) and added to the SPE enriched environmental water sample. The concentrations were 10 µM in both cases. All samples were analyzed three times. Differences between different conditions were calculated by two-sample t-test.

					RPLC-HILIC/TOF-MS						SFC/TOF-MS				
	Compound Name	**Empirical Formula**	**Monoisotopic Mass**	**log D (pH 7)**	**Signal area standard conditions**	**SD**	**Signal area in spiked water sample**	**SD**	**p-value**		**Signal area standard conditions**	**SD**	**Signal area in spiked water sample**	**SD**	**p-value**
Positive ESI mode	Aminomethyl propanediol	C4 H11 N O2	105.0794	-4.68	1.30E+07	4.00E+05	5.80E+06	4.80E+05	0.000		9.10E+05	7.50E+04	1.80E+06	3.80E+05	0.033
	Ethyl 2-oxopyrrolidine-1-acetate	C8 H13 N O3	171.0895	-0.38	1.40E+06	6.20E+04	1.10E+06	5.30E+04	0.006		2.20E+06	2.30E+05	2.50E+06	3.00E+05	0.280
	Oxaceprol	C7 H11 N O4	173.0688	-2.96	3.40E+05	1.20E+04	2.90E+05	7.20E+04	0.347		2.00E+06	3.40E+05	3.30E+05	1.20E+05	0.003
	Molinate	C9 H17 N O S	187.1032	2.34	7.80E+05	3.30E+04	1.90E+05	2.10E+04	0.000		1.50E+05	1.50E+04	2.20E+04	1.10E+04	0.001
	Acetyllysine	C8 H16 N2 O3	188.1158	-3.15	1.20E+07	6.10E+05	1.80E+06	1.80E+05	0.000		3.10E+06	4.70E+05	2.00E+05	6.60E+04	0.001
	Minoxidil	C9 H15 N5 O	209.128	-2.68	1.70E+07	1.90E+05	8.50E+05	2.00E+04	0.000		1.50E+07	1.30E+06	2.10E+07	8.50E+05	0.004
	Atrazine	C8 H14 Cl N5	215.0944	2.19	2.70E+07	1.20E+06	4.80E+05	3.40E+04	0.000		1.40E+07	1.10E+06	2.30E+06	1.70E+06	0.002
	Chloridazon	C10 H8 Cl N3 O	221.0357	1.11	1.10E+07	2.90E+04	3.80E+05	2.40E+03	0.000		8.80E+06	8.20E+05	6.70E+06	7.70E+05	0.056
	Tetraglyme	C10 H22 O5	222.1464	-0.06	1.60E+07	3.50E+05	1.60E+07	1.60E+06	0.819		1.70E+07	1.70E+06	1.60E+07	7.30E+06	0.950
	Metconazole	C17 H22 Cl N3 O	319.1463	3.59	3.30E+07	1.90E+06	4.20E+05	6.00E+04	0.000		2.50E+07	2.40E+06	3.80E+06	2.90E+06	0.001
Negative ESI mode	Oxaceprol	C7 H11 N O4	173.069	-2.96	4.60E+05	9.00E+04	5.80E+05	1.00E+04	0.136						
	Acetyllysine	C8 H16 N2 O3	188.1158	-3.15	1.30E+06	2.70E+05	3.60E+05	5.20E+03	0.007						
	Atrazine	C8 H14 Cl N5	215.0952	2.19	1.60E+04	1.20E+04	4.80E+03	6.50E+02	0.228						
	Chloridazon	C10 H8 Cl N3 O	221.0346	1.11	1.50E+06	9.60E+05	2.50E+05	3.70E+04	0.131						
	Metconazole	C17 H22 Cl N3 O	319.1456	3.59	4.10E+06	2.20E+06	1.80E+06	9.00E+04	0.217						

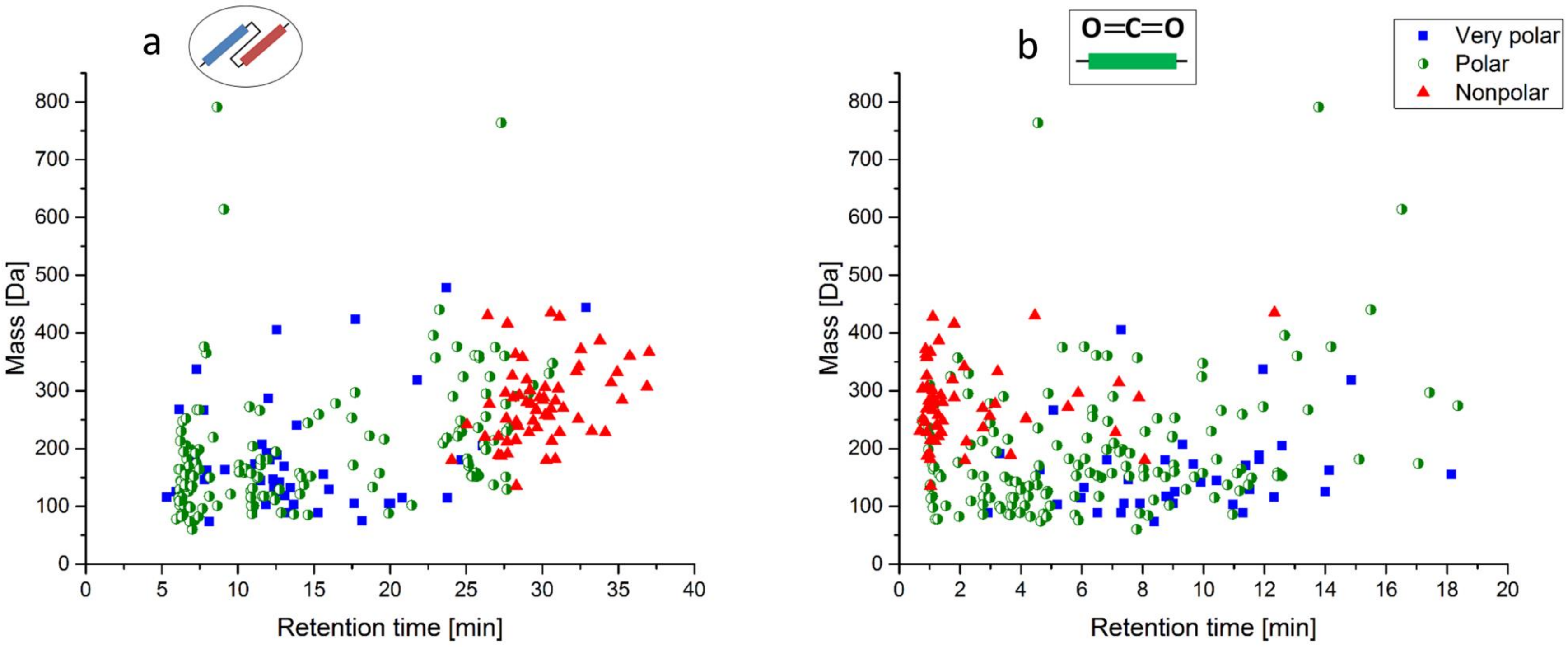

Figure S- 1: RT – mass plots of standard compounds analyzed by RPLC-HILIC/TOF-MS (a) and SFC/TOF-MS (b). Very polar compounds (log D <-2.5, blue rectangles) are mainly retained by HILIC in the RPLC-HILIC coupling, while non-polar compounds (log D > 2.0, red triangles) are exclusively retained by RPLC. Polar compounds are retained in both, HILIC and RPLC, but retention in HILIC seems to be more likely with increased polarity. In SFC (b), non-polar compounds are retained less than very polar compounds. The retention patterns in the RPLC-HILIC coupling (a) shows two groups that represent HILIC- (RT < 16 minutes) and RPLC- retained compounds (RT > 16 minutes).

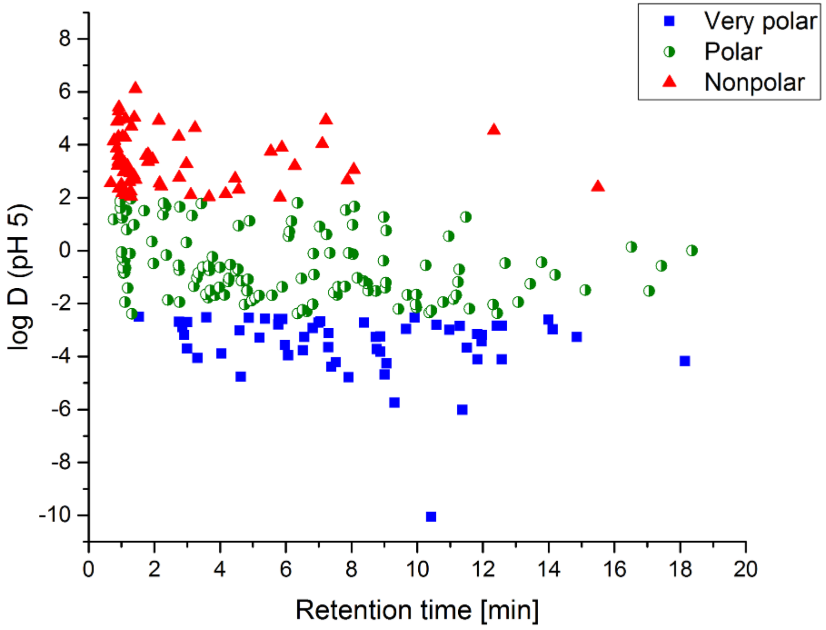

Figure S- 2: RT – log D (pH 5) plot of standard compounds analyzed by SFC/TOF-MS. Also with respect to the conditions of the mobile phase in SFC separations, close to pH 5, a correlation of RT and compound polarity cannot be verified

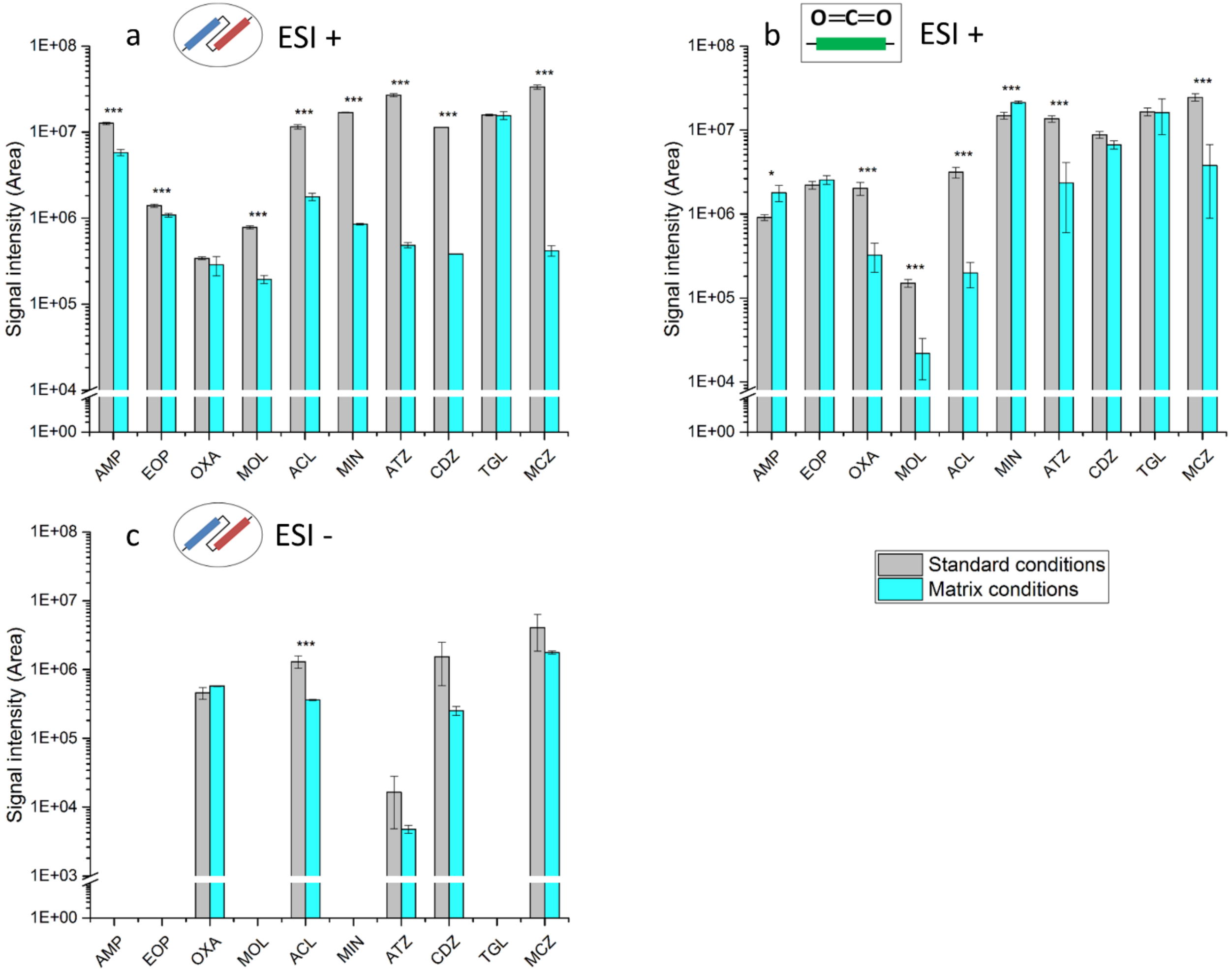

Figure S- 3: Comparison of signal areas obtained from analyses under standard conditions (compounds dissolved in ACN/water (50/50, v/v)) and matrix conditions, where compounds were added to a SPE enriched environmental water sample. The utilized compounds were aminomethyl propanediol (AMP), ethyl 2-oxopyrrolidine-1-acetate (EOP), oxaceprol (OXA), molinate (MOL), acetyllysine (ACL), minoxidil (MIN), atrazine (ATZ), chloridazon (CDZ), tetraglyme (TGL), metconazole (MCZ) and covered a polarity range from -4.68 to 3.59 (Table S-8). Differences between different conditions were determined by two-sample t-test and are marked by asterisks. (***) indicates p-values < 0.01 and (*) indicates p-values < 0.05. In RPLC-HILIC/TOF-MS compounds were detected in positive (a) and negative ESI mode (c), while in SFC/TOF-MS, compounds were only detected in positive ESI ionization mode (b). Y-axis are in logarithmic scale

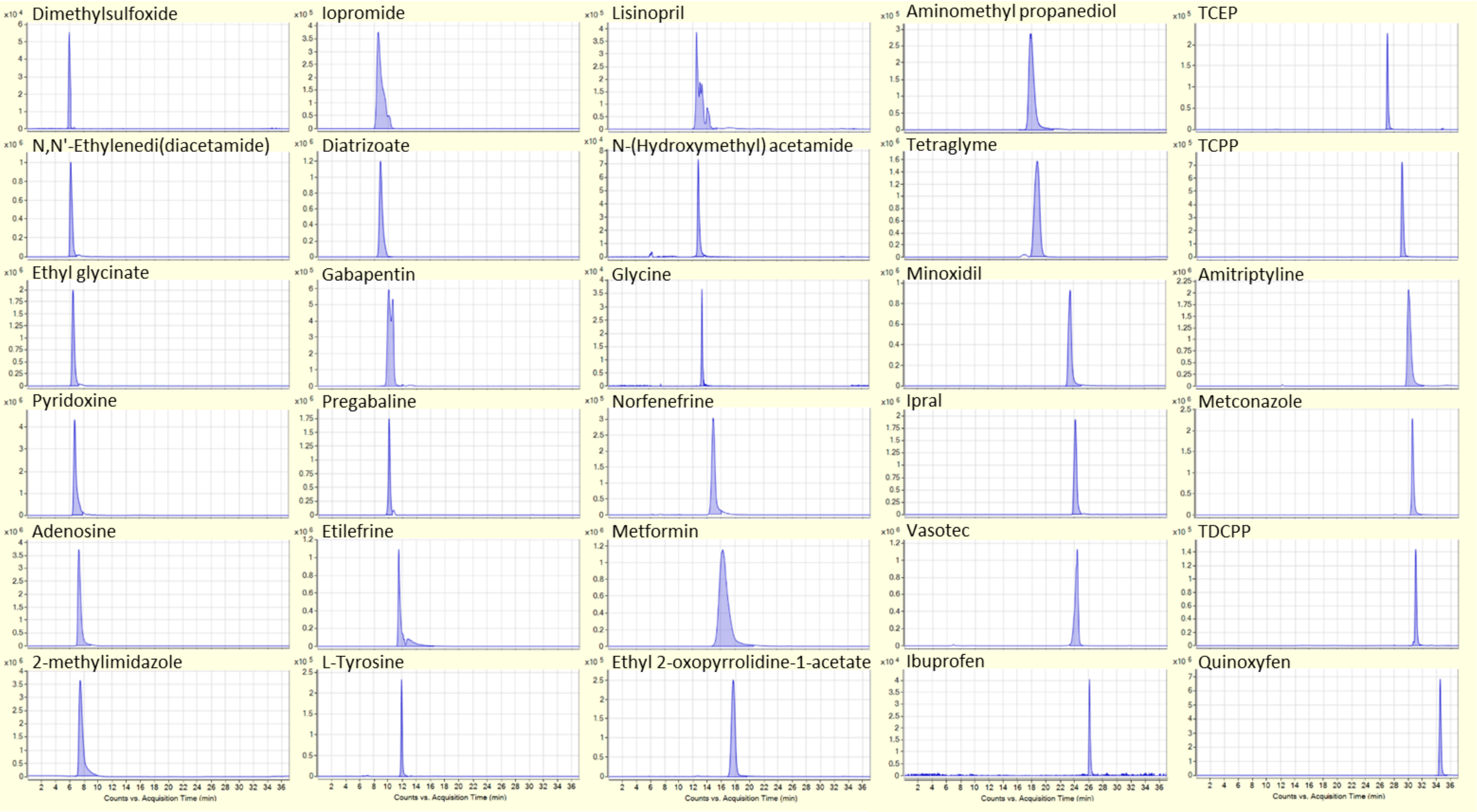

Figure S- 4: MS features of 30 reference standards, separated and detected by RPLC-HILIC/TOF-MS. These features contain extracted ion chromatograms of all detected adducts of a compound.