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Method development for analysis of pharmaceuticals in environmental samples

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Method development for analysis of pharmaceuticals in environmental samples

by

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Abstract

Since the last decades the increasing currency of pharmaceuticals in the aquatic environment and their risk potential for the aquatic life has become an urgent issue. The number of pharmaceuticals detectable in the low µg/L range in the environment is rising each day. Against this background the aim of this project was the development, optimization, validation and comparison of analytical methods for quantification of selected pharmaceuticals in different environmental matrices (water, sediment, suspended particular matter and biota). High sensitive LC-MS-MS methods for determination of extreme polar, polar and hormonal pharmaceuticals in water samples were developed and validated. Using these methods occurrence and distribution of the selected pharmaceuticals in water with different waste water content were determined. For analysis of pharmaceuticals in particular matter and biota samples extraction and clean-up procedures were tested, but method development is still in process. Furthermore a monitoring campaign was set up and water samples, suspended particular matter and biota (fish) samples from six different sampling sites were taken.

Kurzbeschreibung

Die Verbreitung von Arzneimittelrückständen in Gewässern und deren Gefährdungspotential für Wasserlebewesen hat in den letzten Jahren beunruhigende Ausmaße angenommen. Immer mehr Arzneimittelwirkstoffe werden in Konzentrationen bis in den unteren µg/L-Bereich in der aquatischen Umwelt nachgewiesen. Vor diesem Hintergrund ist das Ziel dieses Projekts analytische Nachweisverfahren und Probenahmekonzepte für ausgewählte Arzneimittelwirkstoffe und verschiedene Matrizes (Wasser, Sediment, Schwebstoff, Biota) zu entwickeln, optimieren, validieren und vergleichen. Leistungsstarke LC-MS-MS Methoden wurden für den Nachweis von extrem polaren, polaren und hormonellen Wirkstoffen in Wasserproben entwickelt und validiert. Mit Hilfe dieser Methoden wurden anschließend das Vorkommen und die Verteilung der ausgewählten Arzneimittelwirkstoffe in Wasserproben aus Gewässern mit unterschiedlichen Abwasseranteilen ermittelt. Für die Analytik von Schwebstoff- und Biotaproben wurden Extraktions- und Aufreinigungsverfahren getestet, allerdings befinden sich diese Methoden noch in der Entwicklung. Des Weiteren wurde ein Monitoringskonzept entwickelt und Wasser-, Schwebstoff und Biotaproben aus sechs verschiedenen Gewässern entnommen.

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Abbreviations

BfG	Bundesanstalt für Gewässerkunde
CE	Collision Energy
DP	Declustering Potential
ESI	Electrospray ionization
EWG	Einwohnergleichwerte
GPC	Gel permeation chromatography
HILIC	Hydrophilic Interaction Liquid Chromatography
HPLC	High Performance Liquid Chromatography
IGB	Leibniz-Institut für Gewässerökologie und Binnenfischerei
MRM	Multiple Reaction Monitoring
MS	mass spectrometry
MS-MS	tandem mass spektrometry
PSI	Pound-force per square inch
QuEChERS	Quick, Easy, Cheap, Effective, Rugged, Safe
SPE	Solid Phase Extraction
UBA	Umweltbundesamt
UPB	Umweltprobenbank des Bundes
WWTP	Waste Water Treatment Plant
XIC	Extracted Ion Chromatogram

Summary

Background

Since the last decades the increasing currency of pharmaceuticals in the aquatic environment and their risk potential for the aquatic life has become an urgent issue. The number of pharmaceuticals detectable in the low µg/L range in the environment is rising each day. One of these is diclofenac whose from UBA suggested EQS is 0.05 µg/L. Elimination rates of most pharmaceuticals are much higher than those of classical persistent environmental pollutants like PAHs or PCBs, but quasi continues emission via waste water treatment plants result in a so called "pseudo-persistence". Therefore a higher prioritization of pharmaceutical as environmental pollutants in the European Water Framework Directive and German Oberflächengewässerverordnung would be desirable. Determination of pharmaceuticals in the aquatic environment is focused on the liquid phase. However, only a few standardized methods exist. The discussed extreme low EQS for endocrinal substances like 0.035 ng/L for 17α-ethinylestradiol in the water phase of surface water are a big challenge for analytical chemistry. Indeed physicochemical parameters of some pharmaceuticals let accumulation in other compartments seem to be likely. So sediment, suspended particular matter and biota could be interesting alternative matrices for monitoring of pharmaceuticals in surface waters.

Aim of this project

The aim of this project was the development, optimization, validation and comparison of analytical methods for quantification of selected pharmaceuticals in different environmental matrices (water, sediment, suspended particular matter and biota). Furthermore the conception of a monitoring program for pharmaceuticals in water systems was sought. This environmental monitoring should give insights into occurrence and distribution of pharmaceuticals in waters systems with different waste water loads. Therefore corresponding water, particular matter and biota samples should be taken and analyzed.

Methods

In a first step a selection of the analytes was done. Selection was based upon physicochemical properties, consumption rate and ecotoxicological risk. During prioritization and categorization 49 pharmaceuticals were selected for monitoring. Due to their ecotoxicological high risk potential the endocrine active substances of glucocorticoids, mineralcorticoids, and progestogens have been identified as important substances for environmental monitoring where information distribution in the environment is lacking.

To get an overall overview over the occurrence of pharmaceuticals in surface water a suspect-screening method was established. A database with over 1000 entries of potentially relevant substances was used for a systematically suspect-screening of a set of water samples from the river Rhine and Teltow Canal. 97 pharmaceuticals were identified in the analyzed samples.

Pharmaceuticals occur in the environment in trace concentrations (sub µg to pg/L). Therefore high-performance sample preparation technics and high-sensitive quantification methods are required. Using liquid chromatography coupled to mass spectrometry sensitive methods for quantification of the analytes in water samples were developed. Because of the large polarity and concentration range of the analytes separate methods for extreme polar, middle polar and hormonal pharmaceuticals are required.

For chromatographic separation of extreme polar pharmaceuticals a HILIC method was developed. For HILIC the samples have to be dissolved in an organic solvent (acetonitrile). Therefore freeze drying followed by re-dissolution in acetonitrile was used for sample preparation. Middle polar pharmaceuticals were analyzed by RP-HPLC using direct injection of the water samples. Due to the low concentrations for analysis of hormonal pharmaceuticals an enrichment step is necessary. Several hundred milliliters of water sample were concentrated by a solid phase extraction and extracts were purified by a silica clean-up. Using this procedure extreme low limits of detection could be realized.

Sample preparation is much more complex for sediment/suspended particular matter and biota than for water samples. Different extraction and clean-up methods were tested. Method development is still in progress and will be finalized in a follow-up project.

Validation results

The new developed methods were successfully validated. For each method the performance parameter limit of detections, recoveries and reproducibility were tested. The sensitivity of the methods is sufficient enough for environmental monitoring of pharmaceuticals and satisfying recoveries and reproducibility permit reliable analysis results. In first results several pharmaceuticals could be determined in water samples by these methods.

Monitoring campaign

A concept for a monitoring campaign was compiled. Sample sites with different treated wastewater affection have been chosen. Sampling comprises water, sediment/suspended particular matter and biota samples. First samples have been taken and first analysis results reveal occurrence of many of the selected pharmaceuticals in the surface waters. Especially surface waters with high treated wastewater affection show high concentrations of pharmaceuticals. The most contaminated sample was taken from the Teltow Canal. With the used methods 89 pharmaceuticals and metabolites could be detected. The highest concentrations were measured for oxipurinol with 12 µg/L and for valsartanic acid with 4.6 µg/L. Even high potential hormonal acting pharmaceuticals like canrenone and triamcinolone acetonide could be found in concentrations up to 10 ng/L in waters highly affected by treated wastewater.

The results of this project clearly demonstrate a widespread occurrence of top priority pharmaceuticals in surface waters especially in those with high treated waste water affection.

Outlook

Beside high-sensitive analytical methods for water samples high-effective sample preparation technics for sediment/particulate matter and biota samples were developed. In a follow-up project these methods will be used for monitoring of pharmaceuticals in the three compartments water, sediment/suspended particulate matter and biota.

Zusammenfassung

Hintergrund

Seit der letzten Dekade ist das stetig ansteigende Vorkommen von Arzneimittelrückständen in der aquatischen Umwelt zu einem dringenden Problem geworden. Täglich wächst die Anzahl an nachweisbaren Arzneimittelwirkstoffen im unteren µg/L Bereich in der Umwelt an. Eines davon ist Diclofenac, für das vom UBA eine UQN von 0,05 µg/L vorgeschlagen wurde. Zwar weisen die meisten Wirkstoffe höhere Abbauraten auf als die eher klassischen persistenten Umweltschadstoffe wie PAKs oder PCBs , allerdings wird aufgrund des quasi kontinuierlichen Eintrages über kommunale Kläranlagen von einer „Pseudopersistenz" gesprochen, da sie permanent in relativ konstanten Mengen nachgeliefert werden. Daher wäre es wünschenswert, wenn Arzneimittelwirkstoffe im Zuge von Priorisierungsprozessen der europäischen Wasserrahmenrichtlinie (WRRL) und der nationalen Oberflächengewässerverordnung eine stärkere Berücksichtigung finden. Bisher war die Analytik von Arzneistoffen in Gewässern hauptsächlich auf die Wasserphase fokussiert, obwohl nur wenige standardisierte Nachweismethoden zur Verfügung stehen. Die teilweise diskutierten extrem niedrigen Umweltqualitätsnormen für hormonell wirkende Verbindungen wie 0,035 ng/L für 17α-Ethinylestradiol in der Wasserphase von Binnengewässern stellt die Analytik vor eine große Herausforderung. Allerdings ist aufgrund ihrer physikochemischen Eigenschaften für bestimmte Arzneimittelwirkstoffe eine Anreicherung in anderen Kompartimenten als der Wasserphase zu vermuten. Daher ist nicht auszuschließen, dass Sediment-, Schwebstoff- oder Biotaproben interessante Alternativen zur Wassermatrix für das Monitoring bestimmter Arzneimittelrückstände in Gewässern darstellen.

Projektziel

Ziel in diesem Projekt ist es zunächst analytische Nachweisverfahren und Probenahmekonzepte für ausgewählte Arzneimittelwirkstoffe und verschiedene Matrizes (Wasser, Sediment, Schwebstoff, Biota)zu entwickeln, optimieren, validieren und vergleichen. Mit Hilfe dieser Methoden wird dann das Vorkommen und die Verteilung der ausgewählten Arzneimittelwirkstoffe in Wasser-, Schwebstoff-, Sediment- und Biotaproben von unterschiedlichen Gewässerstandorten ermittelt. Durch die Analyse von Proben der Umweltprobenbank des Bundes sollen mögliche Zeittrends im Konzentrationsverlauf der Arzneistoffe untersucht und das Potenzial der neuentwickelten Analysenmethoden für das Gewässermonitoring demonstriert werden. Gegen Ende des Projekts sollen auf Basis der gewonnenen Erkenntnisse Empfehlungen für optimale Strategien zum Nachweis von Arzneimittelrückständen mit unterschiedlichen Stoffeigenschaften in Gewässern abgeleitet werden.

Methoden

Im Zuge der Priorisierung und Kategorisierung wurden 49 Arzneimittelwirkstoffe für das im Projekt vorgesehene Monitoringprogramm ausgewählt. Die Auswahl basiert auf physikochemischen Eigenschaften sowie Verbrauchsmengen und ökotoxikologischen Wirkungen. Als Substanzklasse mit besonders hohem Gefährdungspotenzial sind die hormonellen Wirkstoffe insbesondere die Glucocorticoide, Mineral-corticoide und Progestagene sowie Östrogene für das Monitoring vorgesehen.

Um einen Überblick über die Verbreitung von Arzneimittelrückständen in Oberflächengewässern zu bekommen, wurde eine Suspect-Screening-Methode etabliert. Auf Basis einer Datenbank mit über 1000 Einträgen mit potenziell relevanten Substanzen wurde für ein systematisches Suspect-Screening von Wasserproben aus dem Rhein und dem Teltow Kanal verwendet. In den Proben konnten 97 Arzneimittelwirkstoffe identifiziert werden.

Arzneimittelrückstände treten in der Umwelt in Spuren (unterer µg bis in den pg/L Bereich) auf. Daher sind leistungsstarke Probenvorbereitungs- und Quantifizierungsmethoden erforderlich. Auf Basis der Flüssigchromatographie gekoppelt an die Tandem-Massenspektrometrie wurden sensitive Methoden zur Bestimmung der Analyten in wässrigen Proben entwickelt. Aufgrund des großen Polaritäts-

und Konzentrationsbereichs der Analyten wurden getrennte Multimethoden entwickelt, und zwar für die extrem polaren, mittelpolaren und hormonellen Arzneimittelwirkstoffe.

Für die chromatographische Trennung der extrem polaren Arzneimittelwirkstoffe wurde eine HILIC Methode entwickelt. Für diese Art der Chromatographie ist erforderlich, dass die Proben in einem organischen Lösungsmittel (Acetonitril) gelöst vorliegt. Daher wurde die Gefriertrocknung zur Probenanreicherung und zum Lösungsmittelaustausch eingesetzt. Die mittelpolaren Arzneimittelwirkstoffe wurden über eine Umkehrphase nach direkter Injektion der Wasserproben aufgetrennt. Da die hormonellen Arzneimittel in extrem geringen Konzentrationen auftreten, ist eine Probenanreicherung erforderlich. Mehrere hundert Milliliter wurden über eine Festphase aufkonzentriert und die gewonnenen Extrakte über ein Silica-Clean-Up aufgereinigt. Über diese Probenvorbereitung konnten sehr niedrige Nachweisgrenzen erzielt werden.

Probenvorbereitung und –aufreinigung ist für Schwebstoff- und Biotaproben wesentlich komplexer und aufwändiger als für Wasserproben. Verschiedene Extraktions- und Aufreinigungsmethoden wurden getestet. Die Entwicklung der Methoden ist noch nicht abgeschlossen und wird im Zuge eines Nachfolgeprojekts finalisiert.

Validierung

Die Methoden wurden erfolgreich validiert. Für jede Methode wurden die Verfahrenskenngrößen Nachweisgrenze, Wiederfindung und Reproduzierbarkeit bestimmt. Die Nachweisgrenzen sind hinreichend niedrig für das Arzneimittelmonitoring in Umweltproben und die zufriedenstellenden Wiederfindungen und Reproduzierbarkeit der Methoden garantieren belastbare Ergebnisse. In Tests mit verschiedenen Gewässerproben konnten Arzneimittel erfolgreich nachgewiesen werden.

Monitoringkampagne

Ein Konzept zur Überwachung von Gewässern in Bezug auf die Verunreinigung mit Arzneimittelrückständen wurde ausgearbeitet. Die Beprobung umfasst die Kompartimente Wasser, Sediment/Schwebstoff und Biota. Eine erste Probennahme wurde durchgeführt und erste Analysenergebnisse zeigen das Vorkommen vieler der ausgewählten Arzneimittelwirkstoffe in den untersuchten Gewässern. Insbesondere Oberflächengewässer mit hoher Belastung mit behandeltem Abwasser weisen hohe Konzentrationen an den Arzneimittelwirkstoffen auf. Am stärksten mit Rückständen belastet waren Proben aus dem Teltowkanal. 89 Wirkstoffe und Metabolite konnten nachgewiesen werden. Die höchsten Konzentrationen wurden für Oxipurinol (12 µg/L) und Valsartansäure (4,6 µg/L) gemessen. Sogar hoch potente hormonelle Wirkstoffewie Canrenon und Triamcinolon konnten in Konzentrationen von bis zu 10 ng/L in Gewässern mit hohem Anteil an behandeltem Abwasser nachgewiesen werden.

Die Ergebnisse dieses Projekts verdeutlichen eine weite Verbreitung von hoch prioritären Arzneimittelwirkstoffen in Oberflächengewässern. Besonders betroffen sind solche mit Beeinflussung durch behandeltes Abwasser.

Ausblick

Neben den leistungsstarken Methoden für den Nachweis von Arzneimittelrückständen in Wasserproben wurden Probenvorbereitungsmethoden für die Analytik von Sediment-/Schwebstoff- und Biotaproben entwickelt. In einem Nachfolgeprojekt sollen diese Methoden für das Arzneimitelmonitoring in den drei Kompartimenten Wasser, Sediment/Schwebstoff und Biota eingesetzt werden.

1 Introduction

The project was executed from November 2015 until February 2018. The work plan consists of the following work packages:

- Work package 1: Categorization and prioritization
- Work package 2: Method development and conception of a monitoring program
- Work package 3: Environmental monitoring
- Work package 4: Reporting and elaboration of recommendations

In work package 1 a categorization and prioritization of pharmaceuticals was done. 49 high priority pharmaceuticals have been chosen for the environmental monitoring. Additionally steroid hormones were identified as important substance class and integrated into the project. In work package 2 high-sensitive analytical methods for extreme polar and polar pharmaceuticals as well as steroid hormones in aqueous samples were developed. Method development for sediment/suspended particular matter and biota is still in process and could not be finished during this project. However, basic principles could be worked out and optimization and validation of the method will be done in a follow-up project. Environmental monitoring was started in work package 3 and first results for aqueous samples were obtained using the new developed methods. Additional sampling and analysis of sediment/suspended particular matter and biota sample will be part of a follow-up project. Based on the results of this project no recommendations regarding optimal strategies for determination of pharmaceuticals with different physicochemical properties in water systems can be derived. This will be a topic addressed in the follow-up project, too.

2 Work package 1: Categorization and prioritization

In work package 1 categorization and prioritization of the investigated pharmaceuticals have been done. In co-work with the UBA a list of 49 high priority pharmaceuticals has been worked out (ref. Table 1). Compound selection was based upon the following parameters: physicochemical properties (log K_{OW}; charge state at pH 6-9), production volume, ecotoxicity and occurrence in surface water. Aim of this project is the elaboration of recommendations for optimal strategies for quantification of pharmaceuticals with different physicochemical properties in the aquatic environment. Therefore selection was made to cover a big polarity range (logK_{OW} -1.8 – 7.6). Furthermore the list contains compounds which are neutral (e.g. carbamazepine), anionic (e.g. diclofenac), cationic (e.g. metformin) or zwitterionic (e.g. gabapentin) at environmental pH-values (6-9). This compound selection guarantees that the whole range of characteristics of pharmaceuticals is included. To ensure the relevance of the selected compounds in future environmental monitoring campaigns the list has been aligned with a priority list [46] published by the UBA. One-fifth of the substances e.g. diclofenac are classified as high priority despite their high consumption and an eco-toxicological effect (MECmax/PNEC ≥1). Six further are not classified as priority because of a lack of environmental data. But the consumption of these pharmaceuticals is rising, so a future environmental risk cannot be excluded. The rest of 33 pharmaceuticals are not part of the priority list. However, they were chosen for this project, because they are substances with a high sorption tendency like tetracycline or they are often found in surface water samples. Furthermore some substances have been selected because bioaccumulation properties have been reported in literature (e.g. sertraline). Additionally, some lifestyle drugs such as ritalin and sildenafil were included in the list due to their increasing use.

Table 1: List of high priority pharmaceuticals

Name	log Kow	charge	priority	Name	log Kow	charge	priority
Allopurinol	-0.9	neu.		Lapatinib	6.16	pos.	
Amoxicilline	0.87	zwi.		Levetiracetam	-0.6	neu.	(P)
Aripiprazol	4.81	pos.		Mesalazine	0.75	zwi.	(P)
Azithromycin	4.02	pos.		Metamizadole	0.71	neg.	
Benzylbenzoate	3.7	neu.		Metformin	-1.8	pos.	
Bezafibrate	4.2	neg.	P	Methylphenidate	2.28	pos.	
Bisoprolol	1.87	pos.		Metoprolol	1.88	pos.	P
Bosentan	4.16	neu.		Naproxene	3.2	neg.	P
Carbamazepine	2.45	neu.	P	Oxazepam	2.24	zwi.	
Cefuroxime	-0.98	neg.		Paracetamol	0.46	neu.	
Cetirizine	3.32	neg.		Permethrin	6.58	neu.	
Ciprofloxacin	0.28	zwi.	P	Quetiapin	3.49	pos.	

Name	log Kow	charge	priority	Name	log Kow	charge	priority
Citaloprame	3.74	pos.		Roxithromy-cine	2.5	pos.	P
Clarithromycin	3.2	pos.	P	Sartanic acid	3.02	neg.	
Clindamycin	2.16	pos.		Sertraline	5.1	pos.	
Diclofenac	4.51	neg.	P	Sildenafil	2.51	pos.	
Diphenhydra-mine	3.5	pos.		Simvastatin	4.68	neu.	(P)
Doxycyclin	-0.02	neg.		Sulfametho-xazole	0.89	pos.	P
Duloxetin	4.69	pos.		Tadalafil	2.36	neu.	
Erythromycin	2.5	pos.	P	Thyroxine	4.35	zwi.	
Fluoxetin	4.05	pos.		Tramadol	2.51	pos.	
Gabapentine	-1.1	zwi.	(P)	Triclosane	4.76	neu.	
Ibuprofen	3.97	neg.		Valproic acid	2.75	neg.	(P)
Imatinib	3.89	pos.		Venlafaxin	2.7	pos.	(P)
Lamotrigin	2.5	pos.					

pos. = positive; neg. =negative; neu. = neutral; += classified as high priority

Due to the large polarity range of the analytes the use of one multi-method is not possible. Extreme polar analytes (e.g. metformin, n-acetyl-mesalazine) show insufficient retention on commonly used reversed phase columns. Therefore special chromatographic methods such as hydrophilic interaction chromatography (HILIC) are required. So, two different methods have been developed for the extreme polar and the middle polar pharmaceutical, respectively (see chapter 4.1.2 and 4.1.3).

Steroid hormones are widely used in human therapy. Examples are the treatment of inflammatory diseases like rheumatism and asthma or contraception. As a consequence, the number of approved synthetic hormones is still increasing and thus, the loads entering the wastewater treatment plants (WWTPs) are appreciable [54]. Glucocorticoids, for instance, are crucial steroid hormones. Similar to other hormones they are administered as tablets, inhalation-powders, nasal sprays, eye and ear drops, injections, shampoos, creams, ointments, foams or lotions [55, 56]. Since they are partially metabolized, their metabolites are excreted together with the unchanged compounds via urine and feces. Additionally, non-metabolized steroid hormones can be washed off from skin for topically utilized products. Therefore, a mixture of steroid hormones enters the municipal WWTPs. It has to be noted that several natural steroid hormones are also excreted in substantial quantities [57, 58] without medicinal therapies.

If steroidal hormones, or their metabolites, pass the WWTPs they are discharged into the receiving waters. Induced endocrine disruption in wildlife by natural and synthetic steroids has been known for decades [59-63] and hence became an important topic in environmental research. In mammals and fish for instance, endogenous steroid hormones are involved in various essential physiological processes by binding on intracellular steroid receptors. Due to structural similarities the majority of hormones exhibit cross receptor binding affinities, and therefore they can act as agonists or antagonists on different receptor types [64-66]. Despite their importance in many physiological regulations, exog-

enous steroids are known to cause adverse effects on aquatic biota. Multiple effects have been reported so far on fish exposed to steroids in laboratory experiments [65-69] and even on wild fish populations [70-72]. Moreover, various bioactivities and effects in fish were determined in different water bodies by bioassays [73-76]. Once steroids reach rivers and streams, they are likely to impact the endocrine system of aquatic organisms and are known to trigger adverse effects [72, 77]. Most of the research focused on estrogenic and androgenic compounds [61-62, 73], whereas only little is known about the occurrence, behavior and toxicological effects of other widespread steroid hormone classes such as progestogens (PG), mineralocorticoids (MC) or glucocorticoids (GC) [74-99].

In total, 60 target hormones (ref. Table A1) were selected according to i) elevated usage in human therapy, ii) known excreted metabolites, iii) reported potency on aquatic biota, iv) lack of occurrence data in European rivers and streams and v) topically applied hormones such as diester and monoester derivatives. So the most important hormonal pharmaceuticals were included into the method.

3 Work package 2: Method development and conception of a monitoring program

3.1 Method development

For quantification of the top priority pharmaceuticals in environmental samples high sensitive and selective analytical methods are required. State of the art is the use of liquid chromatographic methods with tandem mass spectrometric detection (LC-MS-MS). Complex matrices like sediment/suspended particulate matter and biota samples demand extensive sample preparation for separation of interfering matrix compounds. In work package 2 high-performance methods were developed for each class of analytes and the described matrices.

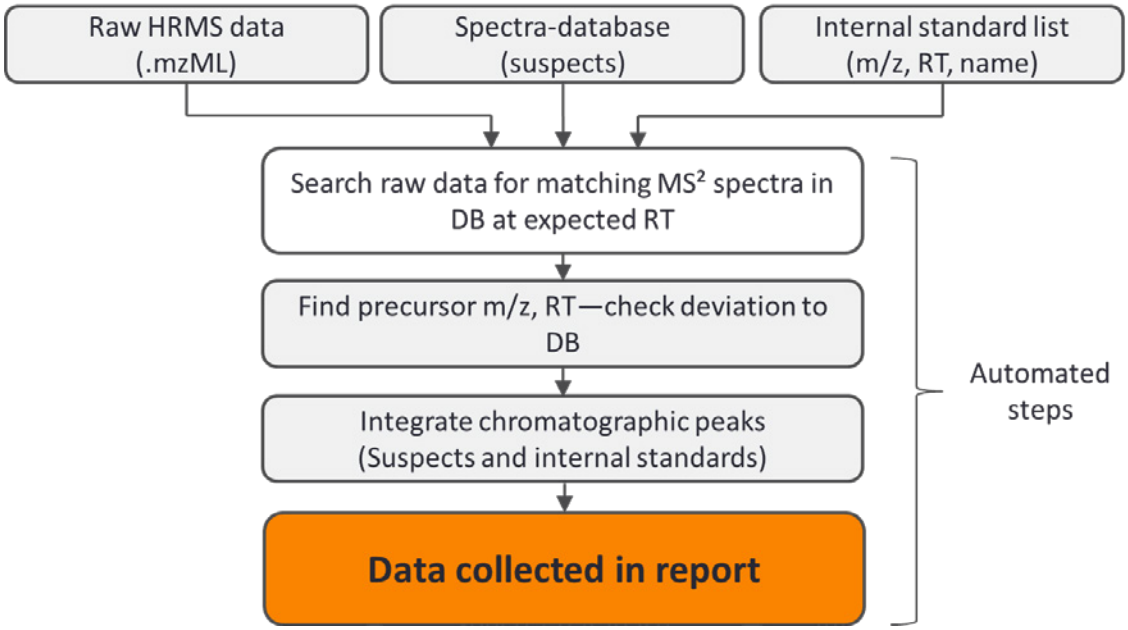
3.1.1 Suspect-Screening

To get a general overview over the occurrence of pharmaceuticals in surface waters a systematic screening method for the identification and trend analysis of a large number of pharmaceuticals, their human metabolites and environmental transformation products was established.

3.1.1.1 Method

Two approaches were run in parallel. In a first trail a database was generated with over 1000 entries of potentially relevant substances, supplemented with chemical formulae, the prescribed amounts in Germany (2014) [38] and reported findings in scientific articles. Based on this data, a suspect-screening was conducted systematically to a set of water samples from the river Rhine and Teltow Canal. For the analysis, a developed in-house LC-HRMS method was used. Details regarding on the analytical method were described in Nuerenberg et al. [121]. The identification of the suspects was achieved by searching the accurate mass-to-charge ratio in the raw data and comparing the MS^2-spectra to online databases (e.g. mzcloud.org, mass-bank.eu). 97 pharmaceuticals (Tab. A3) were identified in the analyzed samples. This time-consuming approach is characterized by a restricted confidence in identification, since no alignment with a reference standard was made. Nevertheless, the benefit was to prioritize compounds of concern for the software supported suspect-screening within the second approach that based on a search of raw data for matching MS^2 spectra in an internal database (Fig. 1).

Figure 1: Scheme of the data processing-steps for the used in-house Suspect-Screening (susS web application) based on the R packages shiny, ggplot2, xcms, metCirc, data.table, dplyr, RSQlite, rcdk, etc.. (Source: Jewell et al. Tracking large numbers of CECs via non-target screening, scientific lecture presented at the "non-target Gewässer kick-off meeting", September 2017, Koblenz, Federal Insitute of Hydrology.)



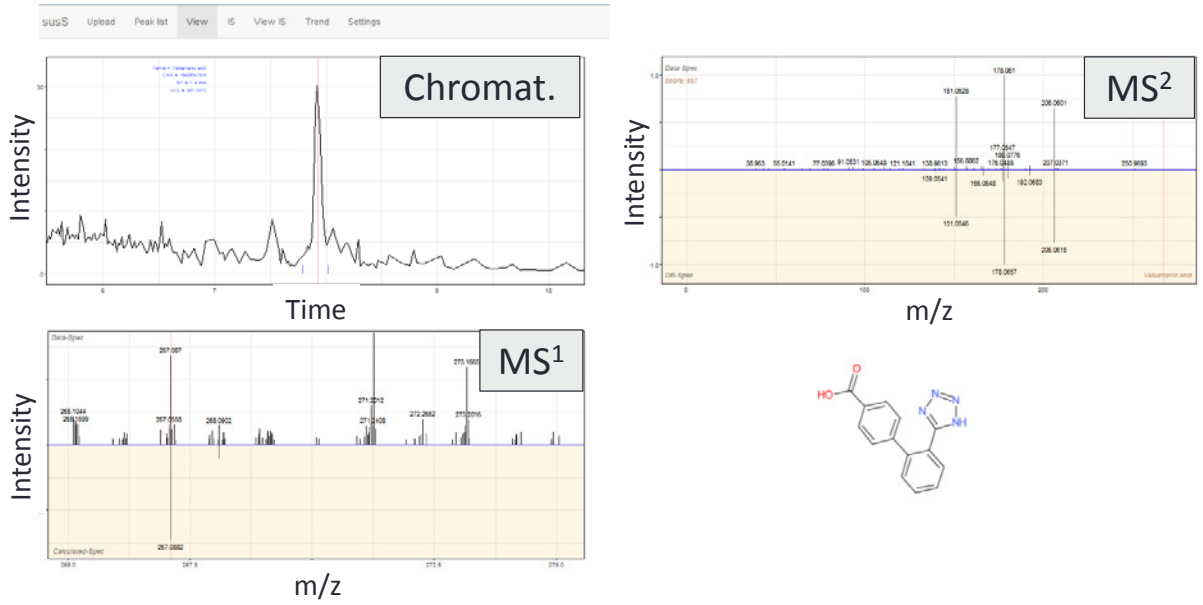
Due to the consideration of the analyte retention time, accurate mass as well as the MS^2 spectra recorded by comparable instrument, the number of false positive detections was decreased [121]. Thus, the confidence in identification is considerable increased. In this approach the data processing was implemented by using the programming language R. The identified suspects can be visualized and corrected immediately via an interactive web applications based on different R packages (Fig. 2).

As shown in Fig. 2, we used the chromatograms of the extracted m/z-values, the MS^1-spectra for comparison of the accurate masses and isotope pattern and finally the MS^2-fragmentation spectra to verify identity of each compound.

3.1.1.2 Results

Water samples from the river Rhine and the Teltow Canal were analyzed. In total 97 pharmaceuticals and metabolites were found. The occurrence of pharmaceuticals and metabolites is much higher in the Teltow Canal which is in line with the higher affection with treated wastewater. Due to the lack of internal standards and calibrations no quantitation could be done. But signal intensities show for some pharmaceuticals a higher content of the metabolites than the mother compounds. This is true for example for torasemide and its metabolite carboxy torasemide. This emphasizes the necessity of monitoring of metabolites. Further results and comparison of the results from suspect screening and target analysis are discussed in chapter 4.3.

Figure 2: Identified suspect (here: valsartanic acid) and its visualization in the interactive web application build in R. Every result is recorded together with chromatogram, MS1 and MS² spectrum. (Source: Jewell et al. Tracking large numbers of CECs via non-target screening, scientific lecture presented at the "non-target Gewässer kick-off meeting", September 2017, Koblenz, Federal Insitute of Hydrology.)



3.1.2 Extreme polar pharmaceuticals (without hormones)

HILIC allows the separation of small polar molecules on a polar stationary phase using water as mobile phase as well as a water miscible organic solvent, most commonly acetonitrile (ACN). Separation mechanisms in HILIC were originally assumed to be based on partitioning between a water-layer coated on the surface of the stationary phase and the less polar mobile phase [1]. However, the mechanism has proven to be much more complex and the involvement of interactions such as sorption, electrostatic forces and hydrogen bonding have also been shown [2-6]. Advantages of HILIC over reversed-phase liquid chromatography (RPLC) include the appreciable retention of polar to extreme polar analytes, low back pressure and higher compatibility with ESI due to elevated organic contents in the eluent [2]. To date, HILIC has rarely been employed for the analysis of environmental samples. Some HILIC methods were applied for the analysis of a given class of molecules: cytostatics [7], antibiotics [8], antidiabetic drugs [9], drugs of abuse [10, 11], organophosphorus pesticides [12] or aromatic amides [13]. However, only few multi-methods exist [14] covering a limited range of compounds with similar polarities. The aim of this study was to develop a high throughput sample pretreatment and a versatile method based on large volume injection HILIC-tandem MS detection for the determination of extreme polar pharmaceuticals as well as their major metabolites and TPs in aqueous environmental matrices including drinking water. The analytes were chosen due to their environmental relevance and to their elevated polarity (Table 2, see Table A3 for structures) so that the results could indicate the applicability of HILIC for environmental analysis. In this framework, the benefits and limitations of HILIC for environmental analysis were evaluated.

Table 2: List of selected analytes, application and log D and charge at pH 7.

Name	Application	CAS No	Formula	Log D at pH 7[1]	Charge at pH 7[1]
4-Acetamidoantipyrine	Metabolite of dipyrone [15]	83-15-8	$C_{13}H_{15}N_3O_2$	0.15	Neutral
4-Formylaminoantipyrine	Metabolite of dipyrone [15]	1672-58-8	$C_{12}H_{13}N_3O_2$	0.11	Neutral
4-Methylaminoantipyrine	Metabolite of dipyrone [15]	519-98-2	$C_{12}H_{15}N_3O$	0.77	Neutral
9-Acridine carboxylic acid	TP of carbamazepine [16]	5336-90-3	$C_{14}H_9NO_2$	0.87	Negative
Abacavir	Antiviral	136470-78-5	$C_{14}H_{18}N_6O$	0.36	Neutral
Abacavir carboxylate	Metabolite of abacavir [17]	384380-52-3	$C_{14}H_{16}N_6O_2$	-2.24	Negative
Acesulfame	Artificial sweetener	55589-62-3	$C_4H_4NO_4S$	-1.49	Negative
Acyclovir	Antiviral	59277-89-3	$C_8H_{11}N_5O_3$	-1.03	Neutral
Bisoprolol	Beta blocker	66722-44-9	$C_{18}H_{31}NO_4$	-0.37	Positive
Clindamycin	Antibiotic	18323-44-9	$C_{18}H_{33}ClN_2O_5S$	0.38	Positive
Clindamycin sulfoxide	Metabolite of clindamycin [18]	22431-46-5	$C_{18}H_{34}Cl_2N_2O_6S$	-1.21	Neutral
Diatrizoate	X-ray constrast medium	737-31-5	$C_{11}H_9I_3O_4$	-0.62	Negative
Emtricitabine	Antiviral	143491-57-0	$C_8H_{10}FN_3O_3S$	-0.90	Neutral
Emtricitabine carboxylate	TP of emtricitabine [17]	1238210-10-0	$C_8H_8FN_3O_4S$	-3.88	Negative
Emtricitabine *S*-oxide	TP of emtricitabine [17]	152128-77-3	$C_8H_{10}FN_3O_4S$	-2.27	Neutral
Gabapentin	Antiepileptic	60142-96-3	$C_9H_{17}NO_2$	-1.27	Zwitterion
Gabapentin lactam	TP of gabapentin [19]	64744-50-9	$C_9H_{15}NO$	1.03	Neutral
Lamivudine	Antiviral	134678-17-4	$C_8H_{11}N_3O_3S$	-1.10	Neutral
Metformin	Antidiabetic	657-24-9	$C_4H_{11}N_5$	-5.69	Positive
Guanyl urea	TP of metformin [20]	141-83-3	$C_2H_6N_4O$	-2.06	Neutral
N-acetyl mesalazine	Metabolite of mesalazine [21]	51-59-2	$C_9H_9NO_4$	-2.26	Negative
Oxipurinol	Metabolite of allopurinol [22]	2465-59-0	$C_5H_4N_4O_2$	-3.03	Negative
Paracetamol	Analgesic	103-90-2	$C_8H_9NO_2$	0.91	Neutral
Ranitidine	H_2 receptor antagonists	66357-35-5	$C_{13}H_{22}N_4O_3S$	0.13	Zwiterion
Desmethyl ranitidine	Metabolite of ranitidine [21]	66357-25-3	$C_{12}H_{20}N_4O_3S$	-0.80	Zwiterion
Ranitidine *N*-oxide	Metabolite of ranitidine [21]	73857-20-2	$C_{13}H_{22}N_4O_4S$	-0.13	Zwitterion
Ranitidine *S*-oxide	Metabolite of ranitidine [21]	73851-70-4	$C_{13}H_{22}N_4O_4S$	-1.17	Zwitterion

3.1.2.1 Material and Methods

Chemicals. LC-MS grade acetonitrile (Lichrosolv®) was purchased from Merck (Darmstadt, Germany). Ammonium formiate (LC-MS grade) was purchased from Fluka Analytics and formic acid (LC-MS grade) was purchased from Sigma-Aldrich (Seelze, Germany). Milli-Q® (18.2 MΩ cm, Merck Millipore, Darmstadt, Germany) was used as ultrapure water. 4-Acetamidoantipyrine, acyclovir, clindamycin, clindamycin sulfoxide, desmethyl ranitidine, emtricitabine, emtricitabine carboxylate, emtricitabine S-oxide, gabapentin, metformin, N-acetyl mesalazine, 4-acetamidoantipyrine-d3, abacavir-d4, acyclovir-d4, bisoprolol-d7 hemifumarate, clindamycin-d3, diatrizoate-d6, emtricitabine-13C,15N2, gabapentin

[1] https://chemicalize.com/

lactam-d6, guanyl urea-15N4 hydrochloride, lamivudine-13C,15N2, paracetamol-d4, acesulfame-d4 potassium salt and oxipurinol-13C,15N2 were purchased from TRC (Toronto, Canada). 4-formylaminoantipyrine, 4-methylaminoantipyrine, abacavir sulfate, acesulfame potassium salt, diatrizoate, gabapentin lactam, N-guanylurea sulfate salt hydrate, oxipurinol, paracetamol, ranitidine hydrochloride, ranitidine N-oxide, ranitidine S-oxide and gabapentin-d10 were obtained from Sigma Aldrich (Seelze, Germany). 9-acridine carboxylic acid was purchased from Santa Cruz, bisoprolol fumarate was purchased from Merck (Darmstadt, Germany). Abacavir carboxylate and lamivudine were obtained from LGC standard (Teddington, UK). Elemental chloride and nitrate standard were purchased from Certipur (Merck, Darmstadt, Germany). Individual stock solution at 1 g/L were prepared for each analyte in methanol or Milli-Q and stored at –25 °C. From these solutions, multi-standard solutions were prepared in acetonitrile.

Sample preparation. Two sample preparation procedures were compared: solid-phase extraction (SPE) and freeze-drying. Different types of adsorbent were evaluated for SPE, Strata XCW (6 mL, 500 mg), Oasis MCX (3 mL, 60 mg), Oasis WCX (6 mL, 500 mg), Oasis HLB (6 mL, 200 mg) and Isolute ENV+ (6 mL, 500 mg). For each cartridge, 100 mL Milli-Q spiked at 0.2 µg/L were enriched and different pH values were tested. Freeze-drying was performed in 15 mL polypropylene centrifuge tubes. Depending on the water matrix, different volumes were used: 10 mL for Milli-Q and groundwater, 5 mL for surface water with a low content in WWTP effluent (< 30 %) and 1 mL for WWTP effluent and for surface water with a high proportion of WWTP effluent (> 30 %). Surrogate standards (0.2 ng) were added to the water samples. Afterwards, the samples were frozen at –25 °C and freeze-dried with a Christ Alpha 2-4 (Christ, Osterode am Harz, Germany). The residues were dissolved by the subsequent addition of 100 µL Milli-Q and 900 µL acetonitrile. The samples were centrifuged for 10 min at 6000 rpm (revolution per minute) with a Hettich Mikro 220R (Tuttlingen, Germany) to eliminate salts precipitated after acetonitrile addition.

HILIC-ESI-MS/MS detection. The LC system consisted of a G1367E autosampler, a G1330B cooling thermostat for the autosampler, a G1312B binary LC pump, a G1310B isocratic LC pump, a G1379B membrane degaser and a G1316A column oven (all Agilent 1260, Waldbronn, Germany). Separation was performed using a zwitterionic HILIC Nucleodur (250 x 3 mm, 3 µm, Macherey-Nagel, Düren, Germany) equipped with a EC HILIC Nucleodur column guard (4 x 3 mm, 3 µm, Macherey-Nagel). The flow rate was set to 500 µL/min. Eluent A was 10 mM ammonium formiate with 0.1 % formic acid and eluent B, 7.5 mM ammonium formiate in acetonitrile/Milli-Q, (90/10, v/v) with 0.1 % formic acid. The following solvent gradient was applied: 0 to 3 min, 100 % B, 3 to 17 min 100 to 75 % B, 17 to 22 min, 75 % B and 22.1 to 33 min 100 % B. The injection volume was 70 µL and the column temperature was set to 25 °C.

For comparison and better understanding of the retention mechanism a Luna HILIC (150 x 3 mm, 3 µm, Phenomenex, Torrance, CA, USA) column was also utilized. The influence of the presence of ammonium formiate in the eluent was tested for both columns. The impact of the pH was also tested with the comparison of the buffers at pH 3.3 (10 mM ammonium formiate, 0.1% formic acid) and at pH 5.8 (10 mM ammonium acetate, 0.005 % CH3COOH). Additionally, the influence of increasing the equilibration time from 11 to 30 minutes was also investigated. These later experiments were performed without divert valve as they were likely to significantly influence the retention times. Mass spectrometric detection was performed using a triple quadrupole mass spectrometer system (QqQ-LIT-MS, API 6500 Qtrap, SCIEX, Darmstadt, Germany) equipped with an IonDriveTM ion source. Electrospray ionization (ESI) with polarity switching was used. The MRM transitions and the substance-dependent parameters are described in Table A5. The following source parameters were used: curtain gas: 35 psi, ion source gas 1: 45 psi, ion source gas 2: 45 psi, source temperature: 500 °C, entrance potential: -10 V (negative mode)/10 V (positive mode), ion spray voltage: –4500 V (negative mode)/5500 V (positive mode). Advanced scheduled MRM was utilized to improve the number of points per peak and thus the reproducibility. Target scan times of 0.5 s in positive mode and 0.3 s in negative mode were applied.

To protect the MS-system, a post-column divert valve (Rheodyne, Darmstadt, Germany) directed the LC flow into the waste from 0.0 to 2.0 min and from 22.0 to 33.0 min. Thus, only substances eluting between 2.0 and 22.0 min were directed to the MS. To compensate the missing flow when the LC flow was discharged into the waste, an additional flow of 150 µL/min Milli-Q/methanol (2/3, v/v) was pumped by an Agilent G1311B quaternary HPLC pump (Agilent). MS data acquisition was controlled with Analyst 1.6.2 (SCIEX). For all compounds two MRM transitions were monitored for quantification and confirmation of the analytes.

Quantification and method performance. The calibration standards were prepared by dilution of the multi-standard solution in acetonitrile/Milli-Q (90/10, v/v). If appropriate internal standards were available, the quantification was carried out by an external standard calibration with internal standard correction (Table A5), otherwise the calibration was solely performed by external standard calibration. A 16-point calibration was performed ranging from the limit of quantification (LOQ) to 20 µg/L for most compounds, while it ranged from LOQ to 200 µg/L for acesulfame, diatrizoate, gabapentin, guanyl urea and oxipurinol due to their elevated environmental concentrations. The quantification was based on a linear regression with 1/x weighting. Data processing was performed by the software MultiQuant™ 3.0.2 (SCIEX).

Instrumental precision was determined by repeated injections of 1000 n spiked groundwater samples on the same day (n=6) and on four different days (n=4).

The accuracy of the method was verified by determining the recoveries at three different concentration levels in 4 water matrices (Milli-Q, groundwater, surface water, WWTP effluent). As no reference water was available that did not contain any of the analytes, the original analyte concentrations were subtracted prior to calculation of the matrix-specific recoveries. Relative recoveries were calculated by normalizing the peak area with the peak area of the respective isotope (D, 13C or 15N) labeled internal standards. The precision of the method (reproducibility) was determined by calculating the 95 % confidence intervals of 3 separately spiked water samples.

Different water matrices were spiked with multi-standard solutions prior to freeze-drying and the lowest spike level at which a signal-to-noise ratio (S/N) > 10 for the transition of quantification and a S/N > 3 for the transition of confirmation were reached was defined as the LOQ. If the water matrix already contained the analytes, the LOQ was estimated by extrapolating the measured concentrations to the one corresponding to a S/N ratio of 10. Instrumental quantification limits (IQL) were determined by diluting the spiked standard solution until signal intensities reach a S/N of 10.

Matrix effects. The matrix effects (ME) were determined according to Matuszewski et al. [23]. The samples were spiked after lyophilisation and the areas were compared with a matrix free standard solution of the same concentration.

$$ME[\%] = \left(\frac{\text{Area of sample spiked after lyophilisation} - \text{Area of non-spiked sample}}{\text{Area of standard}} - 1\right) \times 100$$

Positive values correspond to positive matrix effect (ion enhancement) and negative values indicate negative matrix effect (ion suppression).

For better understanding of the matrix effect, post-column infusion was performed with a 1 mL Hamilton syringe and a syringe pump (Standard Infusion Pump 11, Harvard Apparatus, Holliston, MA, USA) by a flow rate of 10 µL/min. By using a static mixing tee (U-466, Upchurch Scientific, Oak Harbor, WA, USA), the LC flow and the respective analyte flow from the syringe pump were combined and introduced into the ion source of the mass spectrometer.

Environmental monitoring. All water samples were filtered using a GF6 glass fiber filter (Whatman, GE Healthcare, Chicago, USA). The samples were kept in darkness in the refrigerator at 4°C up to 72 h

until sample preparation. Validation was performed with a) grab samples from a groundwater well in Koblenz-Arenberg (28th November 2016) which is mainly free of anthropogenic compounds, b) a 28-days composite sample (31th October to 27th November 2016) from the River Rhine (km 590.3, Koblenz, Germany) and c) grab samples of the effluent from the WWTP Koblenz–Wallersheim (28th November 2016).

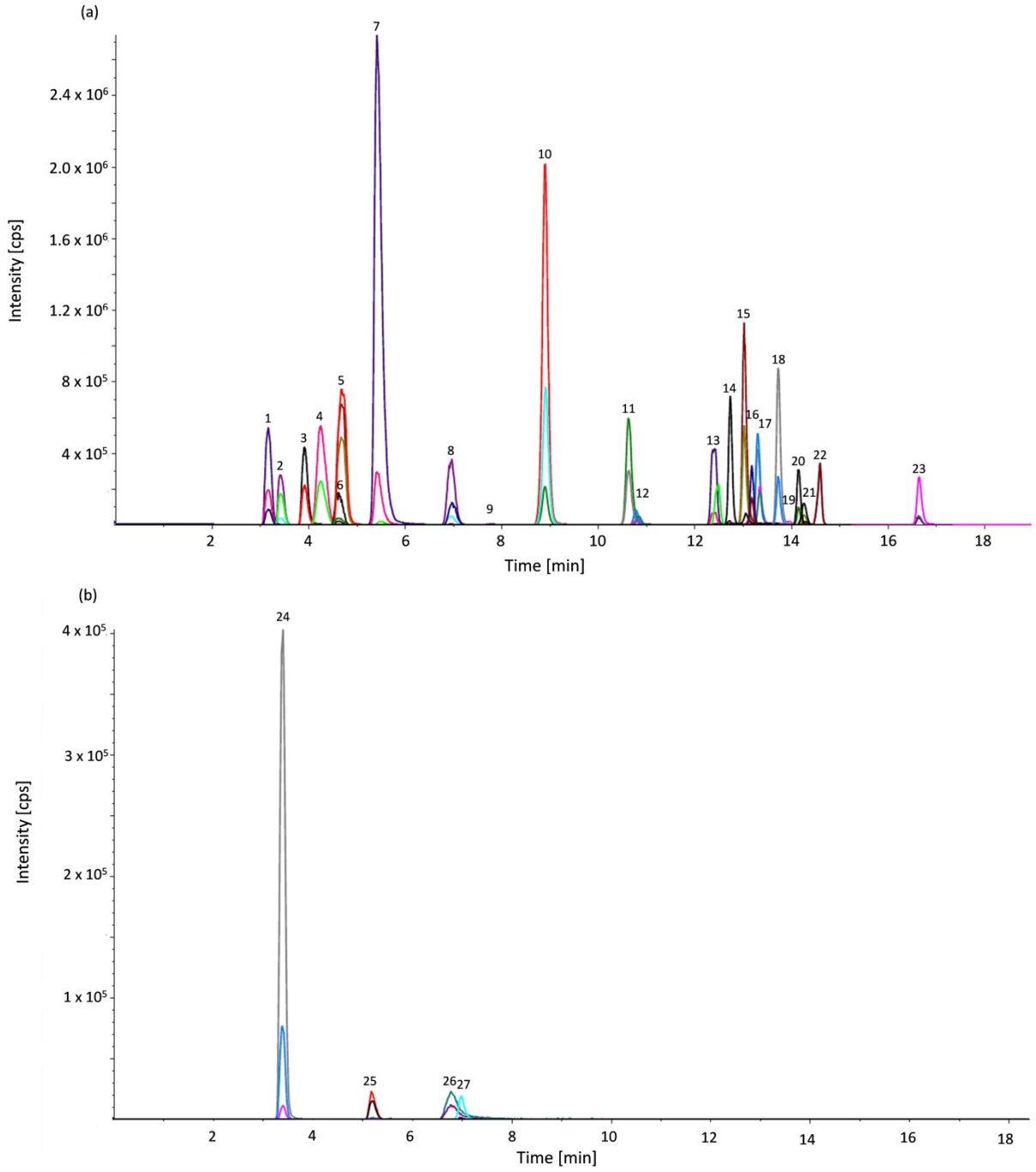
For assessment of the method applicability, drinking water from Germany, groundwater with different origins (region, depth), German rivers and streams with different contents of treated wastewater as well the effluents of two German WWTPs were sampled and monitored.

3.1.2.2 Result and discussion

HILIC-ESI-MS-MS. Chromatographic conditions were optimized on a zwitterionic HILIC Nucleodur. An uncharged stationary phase (Luna HILIC) was applied for comparison. The optimized HILIC-MS/MS method comprises the detection of 26 extreme polar analytes and the artificial sweetener acesulfame. Sufficient retention, very symmetrical peaks (except for N-acetyl-mesalazine all tailing factors were between 0.9 and 1.3) and elevated sensitivities were achieved by adding ammonium formiate buffer and formic acid to both eluents (Fig. 3).

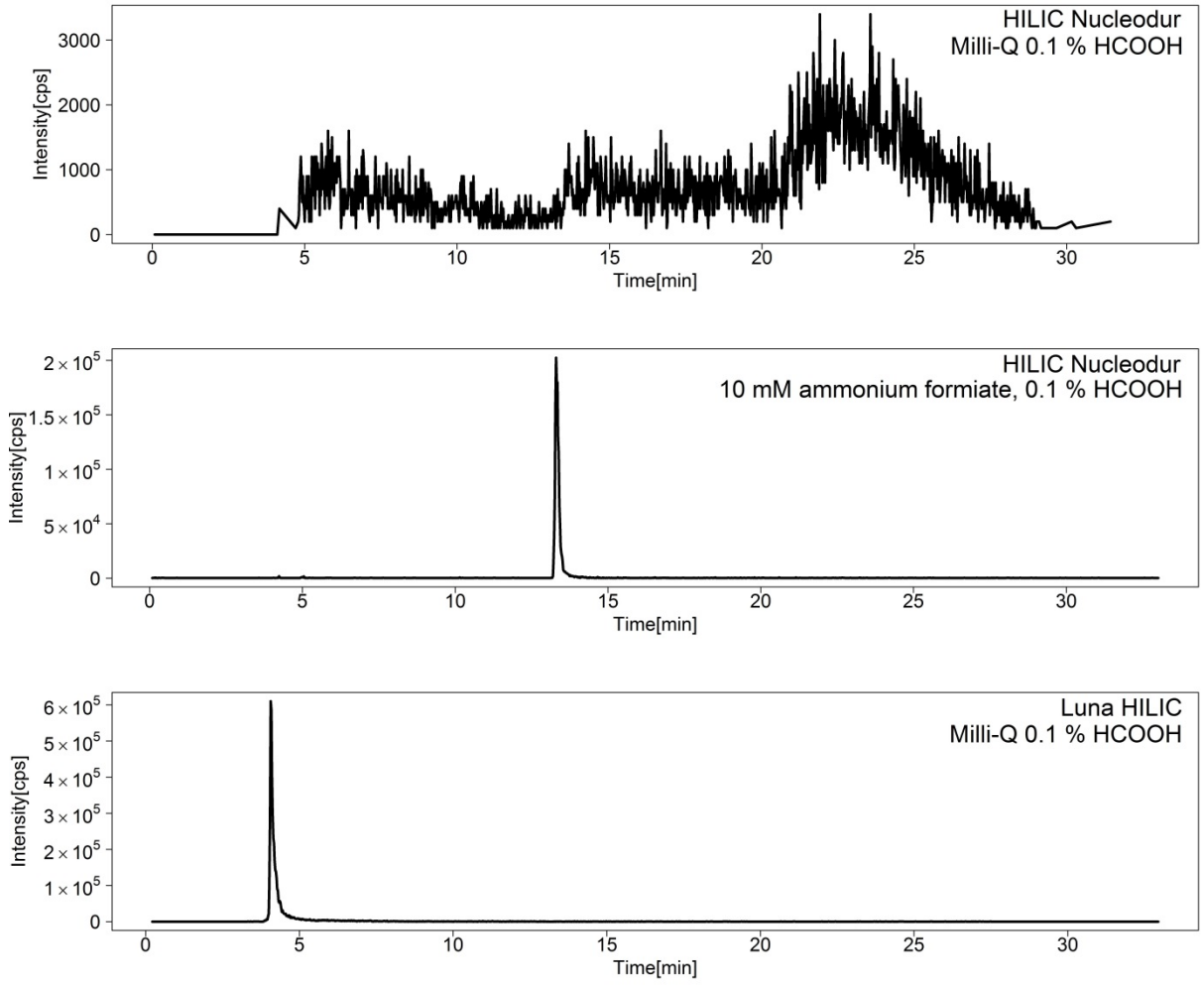
Buffers play a crucial role for HILIC analysis as they influence the electrostatic interactions as well as the thickness and the polarity of the water layer [2, 3]. They are often recommended to ensure the reproducibility of the chromatography [24] and to decrease the interactions of the analytes with the charged stationary phase [24, 25]. In our study, it was found that that the ammonium formiate was essential to ensure a suitable elution of charged analytes on the zwitterionic phase, while for the uncharged stationary phases, such as diol phases (Luna HILIC), this buffer was not required (Fig. 4). This emphasizes that electrostatic interactions are substantially involved in the retention of the charged analytes on the zwitterionic phase. Similarly, addition of ammonium formiate in the organic phase was necessary to ensure appropriate peak forms for 4-methylaminoantipyrine and 4-acetamidoantiantipyrine (see Figure A1).

Figure 3: Superposition of MRM transitions of a 500 ng/L multi-standard. (Source: Own representation, institute of hydrology)



(a) Positive ionization mode. (b) Negative ionization mode. Conditions: HILIC Nucleodur (250 x 3 mm, 3 µm), eluent A (pH 3.3): 10 mM ammonium formiate, 0.1 % formic acid, eluent B: acetonitrile/Milli Q, 90/10, v/v, 7.5 mM ammonium formiate, 0.1 % formic acid, flow rate: 0.5 mL/min, gradient: 100 % B for 3 min, 100 - 75 % B in 14 min, 75 % B for 5 min and 100 % B for 11 min. Detection via HILIC-ESI-MS/MS. Peak identification: (1) gabapentin lactam; (2) paracetamol; (3) 4-methylaminoantipyrine; (4) 4-formylaminoantipyrine; (5) 4-acetamidoantipyrine; (6) emtricitabine; (7) abacavir; (8) lamivudine; (9) emtricitabine S-oxide; (10) bisoprolol; (11) 9-acridine carboxylic acid; (12) acyclovir; (13) clindamycin; (14) ranitidine; (15) gabapentin; (16) desmethyl ranitidine; (17) metformin; (18) ranitidine N-oxide; (19) emtricitabine carboxylate; (20) guanyl urea; (21) diatrizoate; (22) clindamycin sulfoxide; (23) ranitidine S-oxide; (24) acesulfame; (25) oxipurinol; (26) N-acetyl mesalazine; (27) abacavir carboxylate.

Figure 4: Influence of the composition of the aqueous eluent and the column on the elution of metformin. (Source: Own representation, Federal Institute of Hydrology)



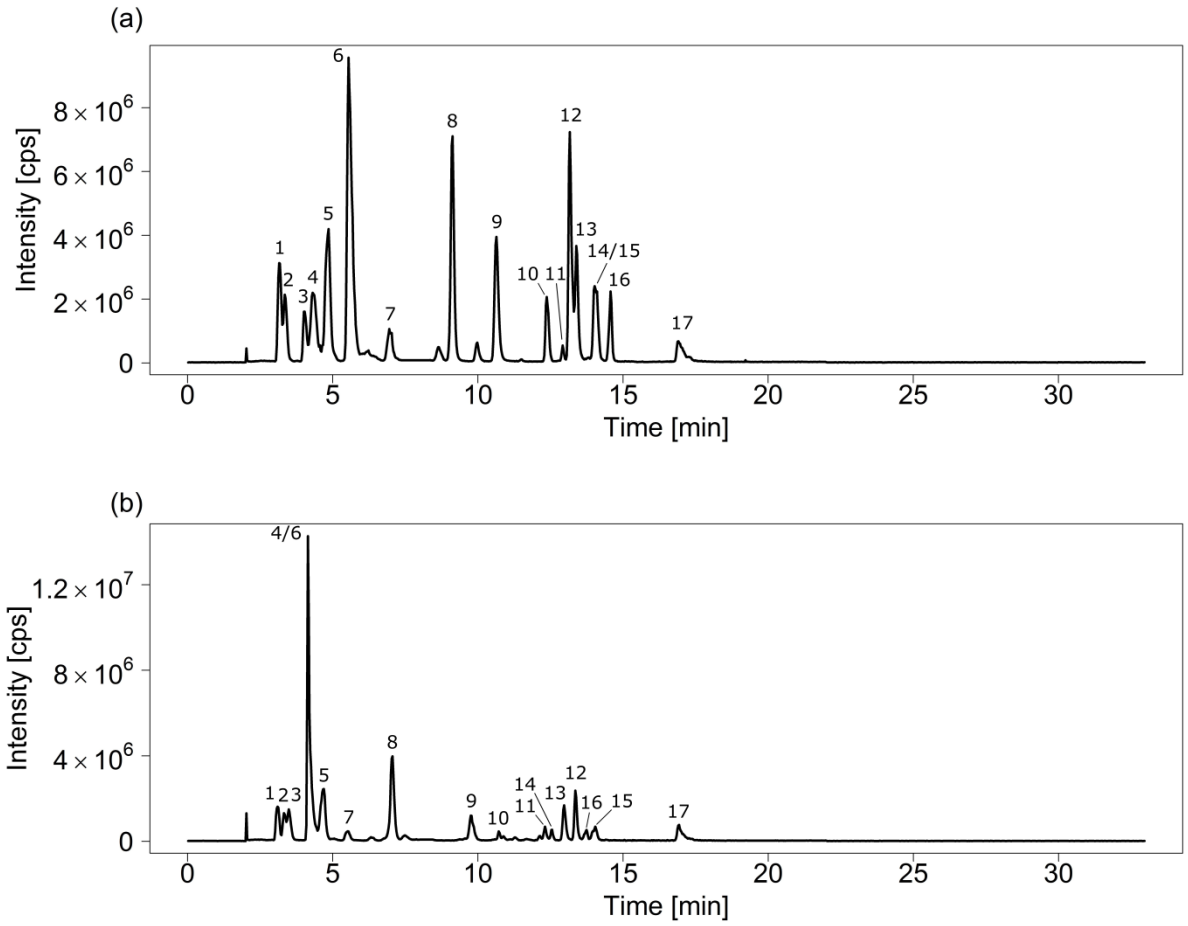
Conditions: (a) column: HILIC Nucleodur (250 x 3 mm, 3 µm), eluent A: 0.1 % formic acid, eluent B: acetonitrile. (b) column: HILIC Nucleodur (250 x 3 mm, 3 µm), eluent A: 10 mM ammonium formiate, eluent B: acetonitrile. (c) column: Luna HILIC (150 x 3 mm, 3 µm), eluent A: 0.1 % formic acid, eluent B: acetonitrile. Detection via HILIC-ESI-MS/MS.

A further important parameter for HILIC analysis is the pH of the mobile phase, which determines the charged state of the analytes, and thus impacts their retention. In some cases, it affects also the charged state of the stationary phase [2, 3]. When the pH of the aqueous eluent was increased from pH 3.3 to pH 5.8, the retention times of positively charged analytes increased significantly (+ 8 min for metformin) making the chromatographic run inappropriately long. The HILIC Nucleodur column is a silica based column and it contains thus an unknown number of silanol groups. At pH > 5, these groups are partially deprotonated increasing their interactions with the positively charged analytes [26-28].

Due to the extended equilibration time needed after the end of the HILIC chromatography run, one recommendation is to use an isocratic elution [29]. However, in our study, the aspired polarity range was too large so that isocratic elution gave unsuitable separation. In order to keep the analysis time as short as possible, the gradient was chosen to be as flat as possible. An equilibration time of 11 min was still essential to ensure reproducible retention times (Table A7) and accurate quantitative results. However, injections directly after extended equilibration duration (30 min) led to a significant shift of the retention times in comparison to 11 min equilibration (Fig. 5). This indicates that the system was not fully equilibrated and illustrates the importance of a compliance of the exact and reproducible

chromatographic conditions as well as the complexity of chromatographic mechanisms occurring in HILIC. We consider however 30 minutes stabilization would have made the method inappropriately long and as long as the retention times are reproducible, there is no need for a complete equilibration of the system between the runs.

Figure 5: Comparison of 2 injections of a 2000 ng/L standard with the HILIC Nucleodur. (Source: Own representation, Federal Institute of Hydrology)



(a) 11 min equilibration. (b) 30 min equilibration. Conditions: column: HILIC Nucleodur (250 x 3 mm, 3 µm), eluent A: 10 mM ammonium formiate, 0.1 % formic acid, eluent B: acetonitrile/Milli Q, 90/10, v/v, 7.5 mM ammonium formiate, 0.1 % formic acid, flow rate: 0.5 mL/min, gradient: 100 % B for 3 min, 100 - 75 % B in 14 min, 75 % B for 5 min and 100 % B for 11 min. Detection via HILIC-ESI-MS/MS.

Sample preparation optimization. For most HILIC columns, the sample extracts have to be injected with a high proportion of organic solvent to enable appropriate symmetric peak shapes [25, 30]. As a consequence, the analysis of aqueous samples is improved by the exchange of water with organic solvents. Hence, the challenge of this work was to find a sample preparation procedure which is suitable for the polarity range applied and the simultaneous analysis of the selected neutral, cationic and anionic substances. In view of this aim, two approaches were compared, i) solid-phase extraction (SPE) and ii) freeze-drying.

In our study, six different SPE cartridges were tested. For each cartridge, the recoveries obtained at 2 to 4 pH values in Milli-Q were investigated. Unfortunately, none of them showed the ability to simulta-

neously retain all or most of the selected analytes (Table 3, see Table A8 for the values). Oasis MCX showed the best results with acceptable recoveries for 13 analytes at pH 3, but very relevant analytes such as metformin or oxipurinol were not retained at all on the cartridge. A combination of several cartridges was also envisaged, but to enrich the whole range of selected analytes, the simplest combination would have involved the combined use of Oasis MCX at pH 3, Isolute ENV+ at pH 8 and Strata XCW at pH 7 (Table A8).

In contrast, freeze-drying showed acceptable recoveries for all analytes ranging from 73 % (lamivudine) to 120 % (9-acridine carboxylic acid). Only ranitidine N-oxide and gabapentin lactam showed lower recoveries with 29 and 50 %, respectively (Table 2) This is probably related to a higher volatility in water with a low salt content, then better recoveries could be obtained in groundwater (see section 3.3). Thus, we decided to use freeze-drying as it represented a more straightforward method than the combination of three different SPE-cartridges at three pH values.

Based on these results, freeze-drying was applied to enable the solvent exchange and a pre-concentration of the analytes. It has already been used for the pre-concentration of antibiotics prior to LC-MS analysis as described in Hirsch et al. [31] but to the best of our knowledge, it is the first time that freeze-drying is used for sample preparation method before HILIC. To limit matrix effects, the freeze-dried water volume was adapted to the water matrix (10 mL for Milli-Q and groundwater, 5 mL for surface water and 1 mL for surface water with a wastewater proportion above 30 % and WWTP effluent).

Redissolving the sample after freeze-drying was achieved by a two-step procedure. First, 100 µL of Milli-Q was added, then the slurry was thoroughly mixed and afterwards 900 µL of pure acetonitrile was added. This simple procedure allowed high throughput, with sufficient recoveries for most selected analytes (see section 3.3).

Table 3 : Recoveries of the analytes with the different investigated sample preparation procedures (see Table S5 for the exact recovery values). (Source: Own representation, Federal Institute of Hydrology)

Analytes	Oasis MCX pH	Oasis MCX pH	Oasis MCX pH	Oasis HLB pH2	Oasis HLB pH3	Oasis HLB pH	Isolute ENV+	Isolute ENV+	Oasis WCX pH	Oasis WCX pH	Strata XCW	Strata XCW	HR-X pH 2	HR-X pH 3	HR-X pH 5	HR-X pH 8	Freeze-Drying
4-Acetamidoantipyrine	2	1	1	1	1	1	2	2	1	1	1	2	1	1	1	1	1
4-Formylaminoantipyrine	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	1
4-Methylaminoantipyrine	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	1
9-Acridine carboxylic acid	1	1	1	3	1	1	4	2	1	1	1	1	1	1	1	1	1
Abacavir	1	1	1	1	1	1	4	1	1	1	1	1	2	1	1	1	1
Abacavir carboxylate	2	1	1	4	4	4	4	2	1	1	1	1	1	1	1	1	1
Acesulfame	4	4	4	4	4	4	4	4	4	4	4	4	3	1	1	4	1
Acyclovir	1	1	1	4	4	4	3	1	3	3	2	2	4	3	2	2	1
Bisoprolol	2	1	1	2	1	1	4	4	1	1	1	1	1	1	1	2	1
Clindamycin	2	1	2	1	1	1	4	4	4	4	3	2	1	1	1	1	1
Clindamycin sulfoxide	1	1	1	1	1	1	4	4	1	1	1	1	1	1	1	1	1
Diatrizoate	4	4	4	2	3	4	1	4	3	4	4	4	1	1	1	3	1
Emtricitabine	2	2	2	4	4	4	4	1	4	2	1	1	3	2	1	2	1
Emtricitabine carboxylate	1	2	3	4	2	4	3	4	4	4	4	4	2	1	3	4	1
Emtricitabine-*S*-oxide	2	1	3	4	4	4	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	4	3	2	2	1
Gabapentin	1	1	1	4	4	4	4	2	1	1	1	1	3	4	3	3	1
Gabapentin lactam	1	1	1	1	1	1	1	1	2	3	2	2	1	1	1	1	3
Lamivudine	1	1	1	4	4	4	4	1	4	3	1	1	4	4	2	2	1
Metformin	4	4	4	4	4	4	4	4	2	2	2	2	4	4	4	4	1
Guanyl urea	1	1	1	4	4	4	4	4	1	1	1	1	n.a.	n.a.	n.a.	n.a.	1
N-acetyl-mesalazine	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	1
Oxipurinol	4	4	4	4	4	4	2	2	4	4	4	4	4	4	4	4	1
Paracetamol	4	2	2	4	2	2	2	2	2	2	1	2	3	1	1	1	1
Ranitidine	2	3	2	3	3	2	4	4	4	4	4	4	3	2	1	2	1
Desmethyl ranitidine	2	3	3	4	4	3	4	4	4	4	4	4	4	3	2	3	1
Ranitidine *N*-oxide	2	3	2	4	2	2	4	4	4	4	4	4	3	2	2	1	2
Ranitidine *S*-oxide	2	3	3	4	4	4	4	4	4	4	4	4	4	4	2	2	1

Recoveries 4: 0-20 %; 3: 20-40 %; 2: 40-70 %; 1: 70-130 %

n.a.: data not available, the analytes were added after SPE experiments were completed

Method performance. The method validation was carried out for four matrices: Milli-Q, groundwater, surface water and WWTP effluent. Five criteria were considered: linearity of the calibration, instrumental precision (repeatability and inter-day precision), accuracy, reproducibility and sensitivity.

For all analytes, the calibration curves showed linear correlation coefficients above 0.99 in the studied range (see Table A9) attesting the good linearity of the analytical method. The instrumental precision was determined by a repeated injection of a 1000 ng/L spiked groundwater on the same day (repeatability, n=6) and on four different days (inter-day precision, n=4). All compounds showed intra-day relative standard deviations (RSD) lower than 20 % indicating a good reproducibility of the detection method (Table 4). For the inter-day precision, only emtricitabine carboxylate showed RSD above 20 %. The lack of an appropriate isotope labeled internal standard is probably the reason of the increased uncertainty for this compound.

To investigate the accuracy and the reproducibility of the method, three spike levels (10, 100 and 1000 ng/L) were examined in Milli-Q and groundwater. In surface water, only 100 ng/L and 1000 ng/L were spiked, since several analyte concentrations already exceeded 10 ng/L. Due to the elevated concentrations, WWTP effluents were only spiked with 5000 ng/L.

For 12 analytes, no isotope labeled standards were available and for metformin and gabapentin a surrogate proton/deuterium exchange occurred during freeze-drying. Consequently, for metformin and those compounds without labeled standards only absolute recoveries without any corrections can be provided.

Most analytes showed acceptable accuracies with recoveries ranging from 80 to 120 % (Table 4). At a spike level of 1000 ng/L in Milli-Q, relative recoveries range from 88 ± 20 % to 134 ± 30 %, in groundwater from 83 ± 13 % to 117 ± 20 % and in surface water from 84 ± 7 % to 134 ± 11 % and in WWTP effluents from 91 ± 23 % to 127 ± 23 %. For most analytes the reproducibility was also acceptable indicated by 95 % confidence intervals below 25 %.

Frequently, even the absolute recoveries were sufficient for quantification. At a spike level of 1000 ng/L in groundwater and surface water, most absolute recoveries varied from 50 to 128 % and from 76 to 125 %. However, for several analytes such as emtricitabine or acyclovir the absolute recoveries reached values sometimes higher than 400 %, probably caused by matrix effects.

Instrumental detection limits ranged from 0.5 ng/L (e.g. 4-acetamidoantipyrine) to 200 ng/L (e.g. oxipurinol) and are thus in the same order of magnitude than in RP-LC multi-residue methods [32]. For most substances, LOQ < 10 ng/L were observed in groundwater and surface water (Table 4). In WWTP effluents, the LOQs were for certain compounds a factor 3 to 5 higher due to the reduced water volume and the elevated matrix. In general, the LOQs are sufficient since most selected pharmaceuticals exhibit in aqueous environmental matrices concentrations in the ng/L to µg/L range.

Table 4: Instrumental precision, instrumental detection limit (IDL) and limit of quantification (LOQ) of the method in the different matrices.

Analytes	Instrumental precision RSD [%]		IQL [ng/L]	LOQ [ng/L]		
	Intra-day (n=6)	Inter-day (n=4)		Ground-water	Rhine Water	WWTP effluent
4-Acetamidoantipyrine	0.9	1.6	0.5	1	1	10
4-Formylaminoantipyrine	1.9	5.4	5	1	2	10
4-Methylaminoantipyrine	1.7	14	1	1	5	20
9-Acridine carboxylic acid	1.8	12	10	1	1	5
Abacavir	3.4	6.9	5	1	5	10
Abacavir carboxylate	2.1	6.3	20	10	10	20
Acesulfame	1.7	1.9	5	1	1	5
Acyclovir	1.0	1.8	10	1	2	50
Bisoprolol	0.3	1.8	2	1	1	2
Clindamycin	4.7	11	0.5	0.1	0.5	2
Clindamycin sulfoxide	3.0	14	5	1	1	5
Diatrizoate	4.7	7.4	5	10	10	50
Emtricitabine	2.1	3.9	10	1	1	5
Emtricitabine carboxylate	9.0	34	10	5	10	50
Emtricitabine *S*-oxide	15	6.2	200	10	50	200
Gabapentin	0.5	8.3	200	50	50	150
Gabapentin lactam	1.1	1.2	5	10	10	20
Lamivudine	1.1	5.5	5	1	5	20
Metformin	1.4	6.0	50	5	5	20
Guanyl urea	1.0	1.7	100	20	20	150
N-acetyl mesalazine	1.0	19	50	10	10	50
Oxipurinol	6.8	4.6	200	50	200	200
Paracetamol	0.9	3.1	20	5	20	250
Ranitidine	1.4	11	0.5	0.1	0.5	0.5
Desmethyl ranitidine	1.3	0.8	1	5	5	5
Ranitidine *N*-oxide	1.8	2.4	20	5	5	5
Ranitidine *S*-oxide	0.9	6.5	10	1	1	5

Table 5: Recoveries and reproducibility of the method (expressed as 95 % confidence intervals).

	Recoveries Milli-Q (n=3) [%]						Recoveries groundwater (n=3) [%]						Recoveries Rhine water (n=3) [%]				Recoveries WWTP effluent (n=3) [%]	
	10 ng/L		100 ng/L		1000 ng/L		10 ng/L		100 ng/L		1000 ng/L		100 ng/L		1000 ng/L		5000 ng/L	
Analytes	Abs	Rel.	Abs	Rel	Abs	Rel	Abs	Rel	Abs	Rel	Abs	Rel	Abs	Rel	Abs	Rel	Abs	Rel
4-Acetamidoantipyrine	101 ± 7	97 ± 3	101 ± 11	97 ± 5	93 ± 1	98 ± 3	102 ± 10	98 ± 4	105 ± 14	98 ± 1	107 ± 13	97 ± 3	112 ± 23	90 ± 11	117 ± 10	93 ± 6	91 ± 7	100 ± 8
4-Formylaminoantipyrine	98 ± 5	94 ± 2	98 ± 15	94 ± 6	89 ± 2	94 ± 2	125 ± 4	120 ± 8	124 ± 16	117 ± 1	128 ± 16	116 ± 3	159 ± 30	127 ± 11	150 ± 8	120 ± 5	113 ± 19	127 ± 22
4-Methylaminoantipyrine	73 ± 13	-	75 ± 24	-	88 ± 9	-	57 ± 15	-	54 ± 91	-	87 ± 24	-	115 ± 10	-	125 ± 12	-	100 ± 3	-
9-Acridine carboxylic acid	110 ± 11	-	104 ± 15	-	104 ± 3	-	119 ± 18	-	121 ± 8	-	128 ± 35	-	117 ± 3	-	115 ± 7	-	69 ± 4	-
Abacavir	104 ± 6	98 ± 2	99 ± 14	97 ± 7	99 ± 8	101 ± 5	127 ± 11	92 ± 5	127 ± 35	96 ± 8	151 ± 45	117 ± 20	127 ± 17	104 ± 3	121 ± 3	103 ± 10	91 ± 8	101 ± 3
Abacavir carboxylate	n.d.	-	73 ± 6	-	100 ± 4	-	n.d.	-	44 ± 13	-	50 ± 16	-	64 ± 4	-	76 ± 2	-	83 ± 6	-
Acesulfame[2]	227 ± 20	102 ± 2	189 ± 14	100 ± 9	107 ± 4	100 ± 12	307 ± 19	101 ± 3	262 ± 11	102 ± 5	111 ± 18	96 ± 18	140 ± 53	107 ± 7	99 ± 3	103 ± 5	109 ± 6	100 ± 7
Acyclovir	67 ± 20	93 ± 9	87 ± 11	124 ± 4	103 ± 2	134 ± 30	221 ± 12	65 ± 2	339 ± 10	96 ± 3	423 ± 204	116 ± 11	355 ± 15	73 ± 4	410 ± 26	84 ± 7	316 ± 11	99 ± 4
Bisoprolol	95.7 ± 0.2	101 ± 1	96 ± 9	101 ± 6	85 ± 4	103 ± 2	84 ± 5	99 ± 1	84 ± 28	102 ± 4	87 ± 8	103 ± 2	95 ± 16	98 ± 2	97 ± 4	100 ± 4	99 ± 6	100 ± 3
Clindamycin	110 ± 3	110 ± 3	112 ± 12	113 ± 7	106 ± 1	113 ± 5	146 ± 23	110 ± 8	148 ± 26	116 ± 14	161 ± 23	116 ± 12	139 ± 9	119 ± 10	137 ± 15	117 ± 9	102 ± 3	99 ± 5

	Recoveries Milli-Q (n=3) [%]						Recoveries groundwater (n=3) [%]						Recoveries Rhine water (n=3) [%]				Recoveries WWTP effluent (n=3) [%]	
	10 ng/L		100 ng/L		1000 ng/L		10 ng/L		100 ng/L		1000 ng/L		100 ng/L		1000 ng/L		5000 ng/L	
Analytes	Abs	Rel.	Abs	Rel	Abs	Rel	Abs	Rel	Abs	Rel	Abs	Rel	Abs	Rel	Abs	Rel	Abs	Rel
Clindamycin sulfoxide	83 ± 2	-	101 ± 11	-	102 ± 3	-	77 ± 6	-	91 ± 15	-	117 ± 40	-	94 ± 24	-	104 ± 4	-	87 ± 22	-
Diatrizoate[2]	104 ± 2	106 ± 4	101 ± 8	105 ± 5	99 ± 8	104 ± 13	82 ± 36	101 ± 27	77 ± 4	107 ± 5	71 ± 16	99 ± 18	106 ± 7	102 ± 2	103 ± 5	99 ± 3	106 ± 28	105 ± 3
Emtricitabine	121 ± 5	93 ± 4	121 ± 13	95 ± 3	93 ± 2	98 ± 4	342 ± 56	93 ± 3	352 ± 29	96 ± 3	324 ± 38	97 ± 7	310 ± 20	96 ± 4	298 ± 23	96 ± 7	239 ± 9	89 ± 20
Emtricitabine carboxylate	85 ± 13	-	97 ± 11	-	106 ± 3	-	68 ± 16	-	73 ± 12	-	82 ± 68	-	118 ± 27	-	120 ± 11	-	89 ± 20	-
Emtricitabine *S*-oxide	95 ± 11	-	113 ± 11	-	110 ± 1	-	92 ± 9	-	104 ± 4	-	109 ± 25	-	99 ± 5	-	93 ± 7	-	95 ± 9	-
Gabapentin[1]	n.d.	-	54 ± 7	-	98 ± 10	-	29 ± 1	-	57 ± 4	-	106 ± 18	-	116 ± 4	-	125 ± 5	-	110 ± 7	-
Gabapentin lactam	15 ± 23	112 ± 9	9 ± 4	108 ± 9	6 ± 1	116 ± 9	79 ± 16	100 ± 8	87 ± 19	102 ± 4	81 ± 51	106 ± 2	91 ± 8	95 ± 5	88 ± 3	103 ± 5	82 ± 4	106 ± 3
Lamivudine	167 ± 6	111 ± 2	155 ± 23	107 ± 7	81 ± 3	101 ± 1	201 ± 9	110 ± 1	197 ± 8	111 ± 3	115 ± 13	104 ± 2	157 ± 7	106 ± 4	113 ± 6	104 ± 4	102 ± 6	106 ± 1
N-acetyl mesalazine	93 ± 16	-	93 ± 5	-	103 ± 9	-	71 ± 16	-	73 ± 8	-	91 ± 46	-	86 ± 10	-	93 ± 6	-	90 ± 13	-
Metformin	77 ± 2	-	101 ± 10	-	97 ± 1	-	55 ± 2	-	109 ± 22	-	116 ± 2	-	139 ± 68	-	112 ± 8	-	106 ± 8	-
Guanyl urea[2]	121 ± 4	144 ± 8	96 ± 19	124 ± 11	88 ± 13	126 ± 24	145 ± 33	126 ± 4	143 ± 2	128 ± 4	116 ± 31	101 ± 3	144 ± 14	111 ± 7	161 ± 9	134 ± 11	97 ± 9	127 ± 23
Oxipurinol[2]	88 ± 37	93 ± 25	93 ± 15	97 ± 18	95 ± 7	88 ± 20	92 ± 28	80 ± 15	110 ± 6	80 ± 9	84 ± 14	83 ± 13	89 ± 22	119 ± 25	81 ± 4	109 ± 10	73 ± 5	91 ± 23
Paracetamol	82 ± 2	94 ± 3	81 ± 10	94 ± 6	86 ± 3	99 ± 5	70 ± 20	95 ± 7	77 ± 2	95 ± 3	76 ± 42	99 ± 3	79 ± 2	96 ± 6	81 ± 7	100 ± 7	65 ± 3	96 ± 19

	Recoveries Milli-Q (n=3) [%]						Recoveries groundwater (n=3) [%]						Recoveries Rhine water (n=3) [%]				Recoveries WWTP effluent (n=3) [%]	
	10 ng/L		100 ng/L		1000 ng/L		10 ng/L		100 ng/L		1000 ng/L		100 ng/L		1000 ng/L		5000 ng/L	
Analytes	Abs	Rel.	Abs	Rel	Abs	Rel	Abs	Rel	Abs	Rel	Abs	Rel	Abs	Rel	Abs	Rel	Abs	Rel
Ranitidine	99 ± 32	-	96 ± 9	-	110 ± 12	-	79 ± 20	-	71 ± 23	-	74 ± 6	-	103 ± 76	-	98 ± 15	-	110 ± 21	-
Desmethyl ranitidine	73 ± 27	-	70 ± 4	-	75 ± 7	-	110 ± 11	-	96 ± 27	-	88 ± 8	-	88 ± 13	-	84 ± 2	-	94 ± 1	-
Ranitidine *N*-oxide	52 ± 4	-	76 ± 2	-	91 ± 2	-	49 ± 1	-	78 ± 13	-	81 ± 14	-	82 ± 4	-	88 ± 3	-	90 ± 6	-
Ranitidine *S*-oxide	92 ± 7	-	88 ± 5	-	103 ± 2	-	102 ± 4	-	102 ± 23	-	122 ± 21	-	95 ± 23	-	108 ± 3	-	102 ± 3	-

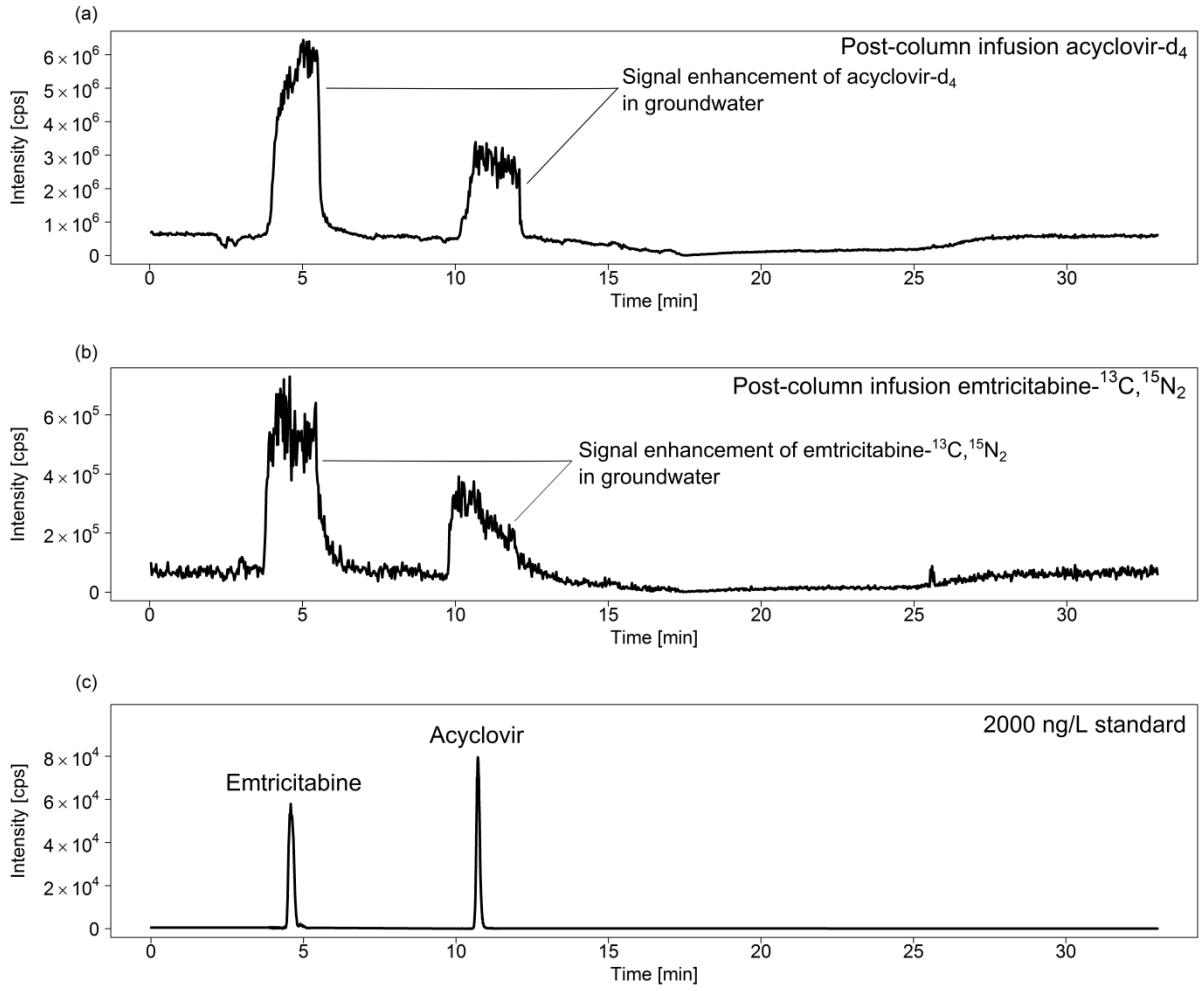
Matrix effects. Matrix effects determined were relatively similar in the different matrices (Table 6). Interestingly, for almost all analytes only positive matrix effects caused by ion enhancement were observed. For some compounds, the positive matrix effects were extremely high as observed for acyclovir or emtricitabine which exhibited matrix effects of above 200 %. Thus, the elevated absolute recoveries reported in the section 3.3. were obviously caused by ion enhancements during ionization.

However, these matrix effects could be compensated by the added internal standards and thus do not hamper quantification (see section 3.3). In order to elucidate the reasons for such elevated ion enhancements, post-column infusion experiments were performed for acyclovir and emtricitabine internal standards (Fig. 6a and b). Appreciable signal enhancements were obtained both for acyclovir and emtricitabine internal standard between 3.8 and 5.6 min and 9.7 and 12 min, i.e. at the retention times of emtricitabine and acyclovir, respectively (Fig. 6c).

Table 6: Matrix effects for the analytes in groundwater, Rhine water and WWTP effluent.

Analyte	Matrix effect [%]		
	Groundwater	Surfacewater	WWTP effluent
4-Acetamidoantipyrine	13 ± 6	21 ± 25	16 ± 9
4-Formylaminoantipyrine	43 ± 5	67 ± 34	55 ± 16
4-Methylaminoantipyrine	-4 ± 6	1 ± 22	-13 ± 9
9-Acridine carboxylic acid	-38 ± 2	9 ± 14	22 ± 4
Abacavir	15 ± 6	37 ± 22	43 ± 7
Abcavir carboxylate	-39 ± 6	18 ± 20	-11 ± 4
Acesulfame	-11 ± 4	-12 ± 19	14 ± 10
Acyclovir	352 ± 8	490 ± 96	472 ± 21
Bisoprolol	10 ± 5	0 ± 15	-7 ± 2
Clindamycin	29 ± 2	76 ± 28	179 ± 29
Clindamycin sulfoxide	3 ± 7	73 ± 33	41 ± 11
Diatrizoate	30 ± 11	36 ± 28	22 ± 5
Emtricitabine carboxylate	20 ± 22	82 ± 53	60 ± 28
Emtricitabine	84 ± 15	220 ± 54	287 ± 6
Emtricitabine S-oxide	0 ± 10	-2 ± 13	0 ± 7
Gabapentin	-7 ± 3	78 ± 16	25 ± 6
Gabapentin lactam	0 ± 8	3 ± 7	-3 ± 6
Lamivudine	18 ± 5	4 ± 10	3 ± 6
N-Acetyl mesalazine	-15 ± 6	15 ± 47	34 ± 12
Metformin	12 ± 3	3 ± 35	-5 ± 10
Guanyl urea	-4 ± 1	-28 ± 10	51 ± 9
Oxipurinol	-44 ± 3	-39 ± 33	-27 ± 10
Paracetamol	-31 ± 4	-16 ± 7	-20 ± 2
Ranitidine	22 ± 2	8 ± 19	22 ± 31
Desmethyl ranitidine	23 ± 12	5 ± 15	7 ± 4
Ranitidine N-oxide	12 ± 3	32 ± 12	15 ± 2
Ranitidine S-oxide	-25 ± 4	13 ± 11	2 ± 5

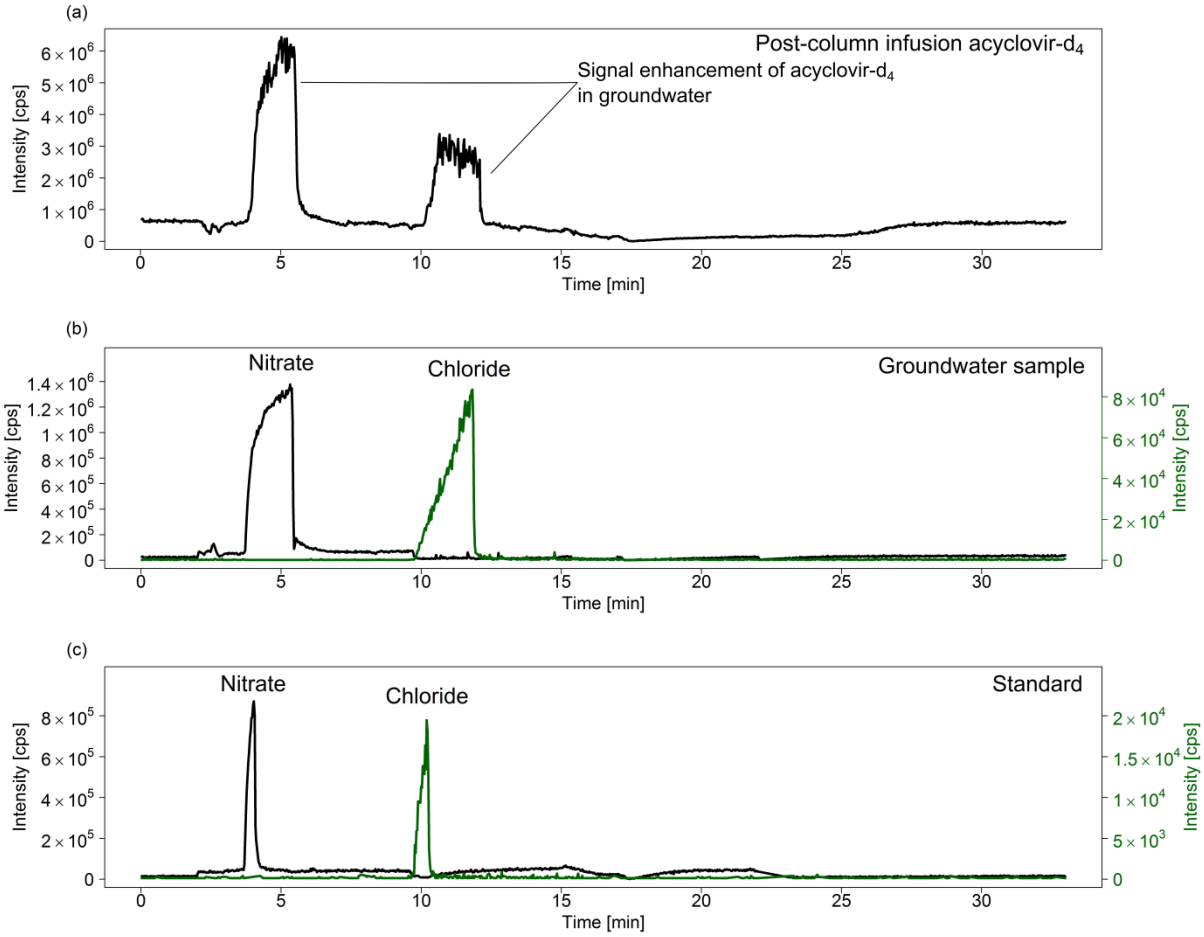
Figure 6: Matrix effects. (Source: Own representation, Federal Institute of Hydrology)



(a). Post-column infusion of 1 mg/L acyclovir-d_4 at 10 µL/min for HILIC-ESI-MS/MS analysis of a groundwater sample. (b). Post-column infusion of 1 mg/L emtricitabine-$^{13}C,^{15}N$ at 10 µL/min for HILIC-ESI-MS/MS analysisof a groundwater sample. (c) Superposition of MRM transitions of emtricitabine and acyclovir of a 2000 ng/L multi-standard. Conditions: column: HILIC Nucleodur (250 x 3 mm, 3 µm), eluent A: 10 mM ammonium formiate, 0.1 % formic acid, eluent B: acetonitrile/Milli Q, 90/10, v/v, 7.5 mM ammonium formiate, 0.1 % formic acid, flow rate: 0.5 mL/min, gradient: 100 % B for 3 min, 100 - 75 % B in 14 min, 75 % B for 5 min and 100 % B for 11 min. Detection via HILIC-ESI-MS/MS.

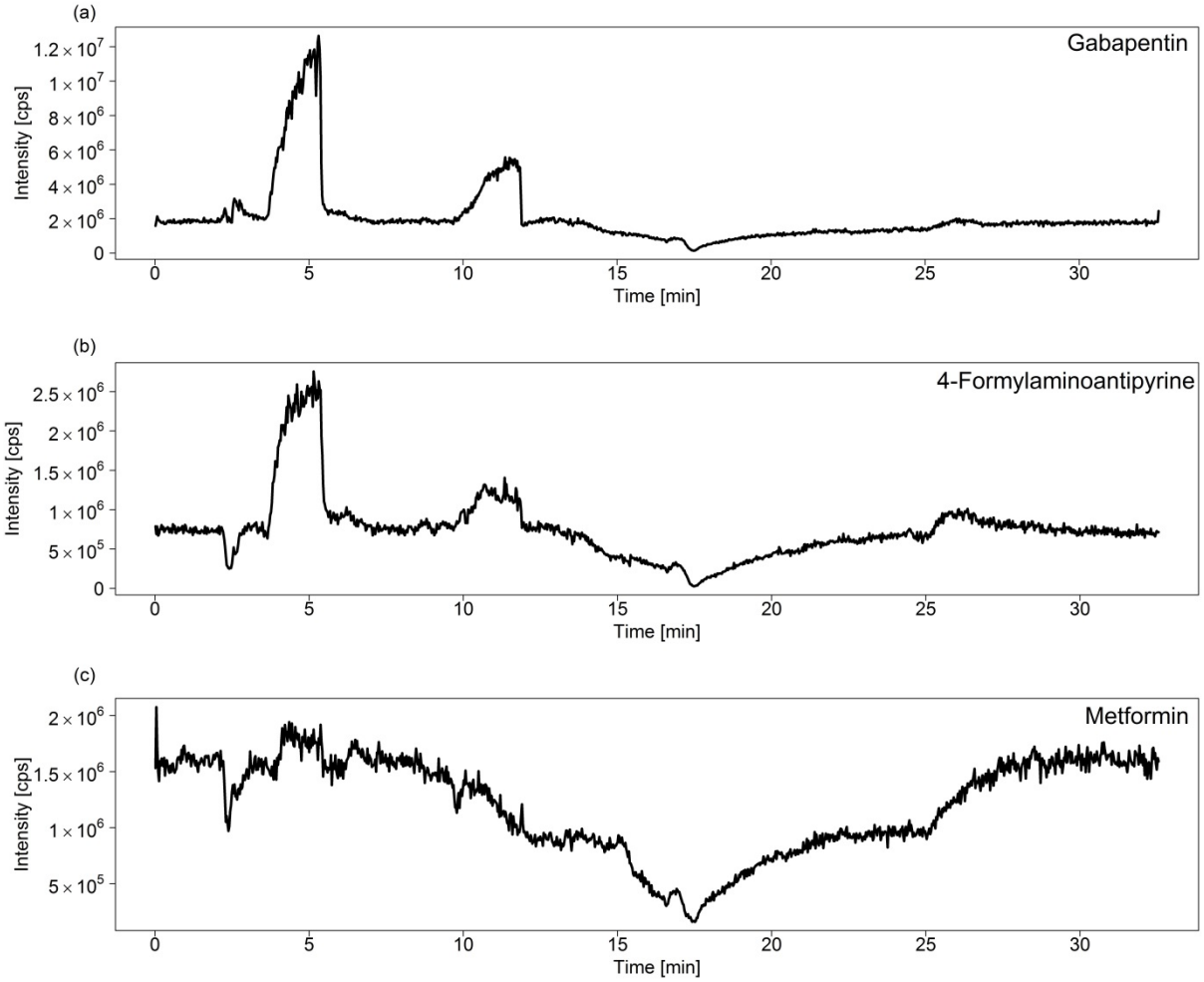
Since the positive matrix effects were observed in all tested matrices even in groundwater, it was hypothesized that they were caused by ubiquitously occurring substances. To test this hypothesis, the analytes were dissolved in omnipresent salt solutions (NaCl, KCl, $NaNO_3$, Na_2SO_4, $CaCl_2$). It was confirmed that the emtricitabine signal was enhanced when nitrate was present and the acyclovir signal was enhanced when chloride was present. LC-MS measurement of nitrate and chloride in groundwater confirmed that signal enhancement windows (Fig. 7a) correspond to the retention time of nitrate and chloride, respectively (Fig. 7b and c, Table A9). Concentration of 0.5 mmol/L in chloride and 0.1 mmol/L in nitrate could be estimated. The post-column infusion of a mixture of the 25 other analytes led to similar effects for 16 of the analytes in the positive mode, albeit with different amplitudes (Fig. 8, (a) and (b)). Several analytes such as metformin did not show any ion enhancement (Fig. 8, (c)).

Figure 7: Post-column infusion of acyclovir-d4. (Source: Own representation, Federal Institute of Hydrology)



(a) Post-column infusion of 1 mg/L acyclovir-d4 at 10 µL/min for HILIC-ESI-MS/MS analysis of a groundwater sample. (b) XIC of nitrate and chloride for a groundwater sample. (c) XIC of nitrate and chloride for a chloride and nitrate mixed 10 mg/L standard. Conditions: column: HILIC Nucleodur (250 x 3 mm, 3 µm), eluent A: 10 mM ammonium formiate, 0.1 % formic acid, eluent B: acetonitrile/Milli Q, 90/10, v/v, 7.5 mM ammonium formiate, 0.1 % formic acid, flow rate 0.5 mL/min, gradient: gradient: 100 % B for 3 min, 100 - 75 % B in 14 min, 75 % B for 5 min and 100 % B for 11 min. Detection via HILIC-ESI-MS/MS.

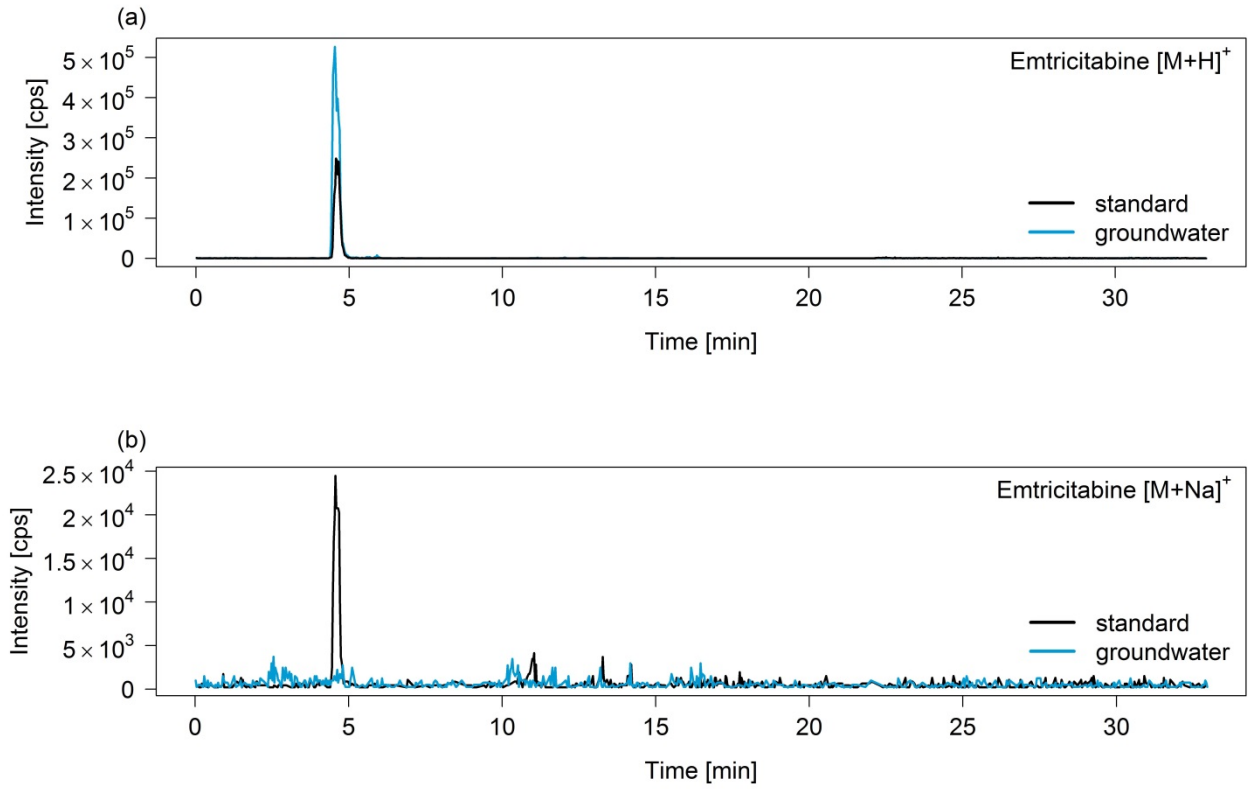
Figure 8: Post-column infusion of a multi-standard. (Source: Own representation, Federal Institute of Hydrology)



Post-column infusion of a 0.1 mg/L multi-standard at 10 µL/min during the measurement of a groundwater sample. (a) XIC of gabapentin. (b) XIC of 4-formylaminoantipyrine. (c) XIC of metformin. Conditions: column: HILIC Nucleodur (250 x 3 mm, 3 µm), eluent A: 10 mM ammonium formiate, 0.1 % formic acid, eluent B: acetonitrile/Milli Q, 90/10, v/v, 7.5 mM ammonium formiate, 0.1 % formic acid, flow rate 0.5 mL/min, gradient: 100 % B for 3 min, 100 - 75 % B in 14 min, 75 % B for 5 min and 100 % B for 11 min. Detection via HILIC-ESI-MS/MS.

Adduct formation is often a source of signal depletion in MS. Sodium adducts, in particular, are ubiquitous and can have dramatic effects on the signal intensities [33]. Iavarone et al. [34] showed that the addition of ammonium acetate to a solution of sodium chloride caused signal improvement due to the decrease of the abundance of the sodium adducts. They hypothesized that NH4Cl and NaCl precipitate because of its lower solubility than ammonium acetate. Although a precipitation was not proven, it is probable that chloride creates ion pairs with sodium and thus its affinity to the analytes is reduced. This would explain the increased signal intensities. A similar phenomenon occurs probably in the presence of nitrate. This was verified for emtricitabine by monitoring of both $[M+H]^+$ and $[M+Na]^+$ in the standard solution as well as in spiked groundwater samples. In the standard solution, the sodium adduct could be clearly identified, while in the groundwater sample it was not detected anymore (Fig. 9). Due to the low tendency of sodium adducts for fragmentation, the sodium adducts of the other molecules could not be analyzed, but a similar behavior can be hypothesized.

Figure 9: Sodium adducts of emtricitabine. (Source: Own representation, Federal Institute of Hydrology)

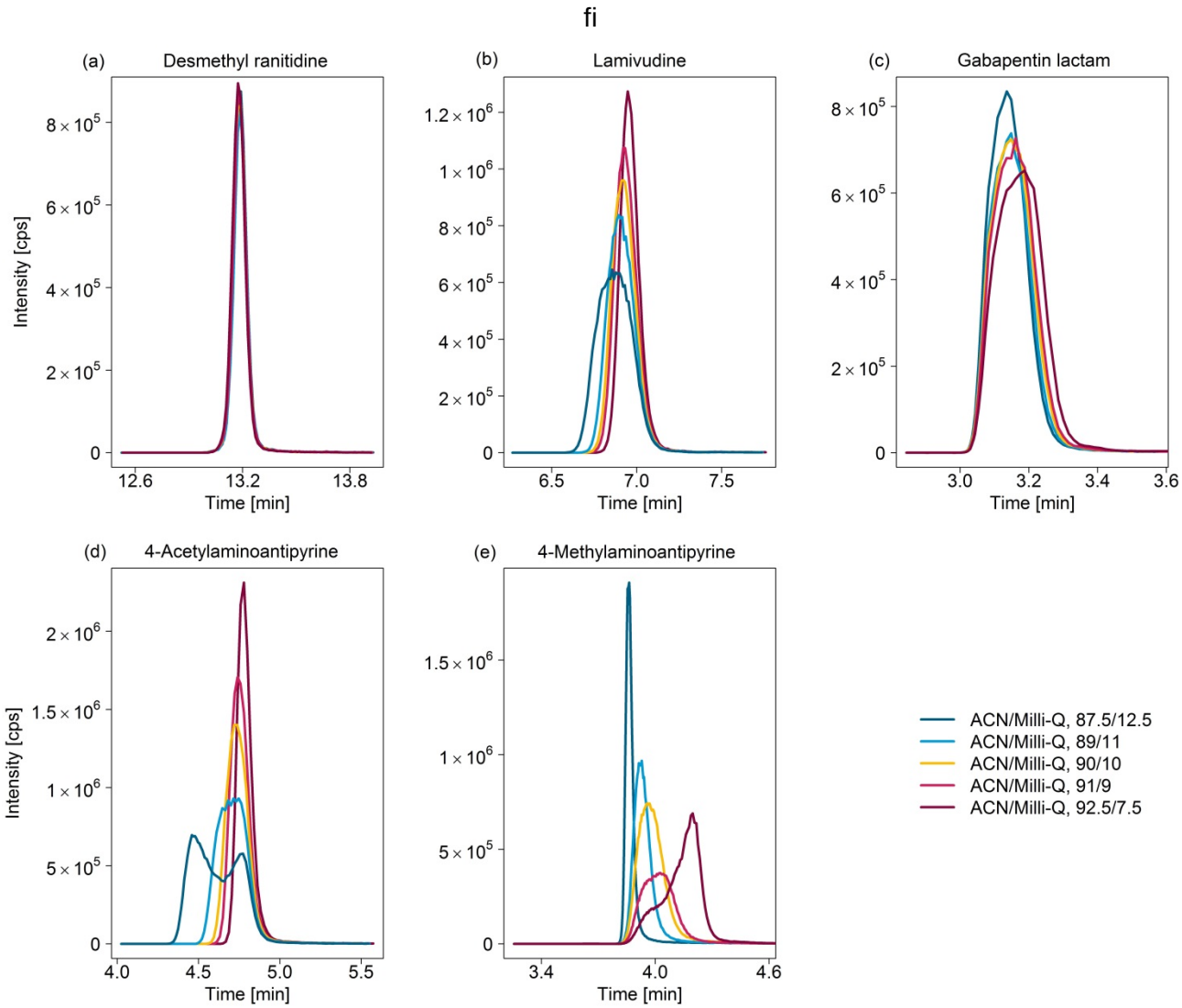


(a) XIC of the protonated ion of emtricitabine for a 1000 ng/L standard and a 1000 ng/L spiked groundwater sample. (b) XIC of the sodium adduct of emtricitabine for a 1000 ng/L standard and a 1000 ng/L spiked groundwater. Conditions: column: HILIC Nucleodur (250 x 3 mm, 3 µm), eluent A: 10 mM ammonium formiate, 0.1 % formic acid, eluent B: acetonitrile/Milli Q, 90/10, v/v, 7.5 mM ammonium formiate, 0.1 % formic acid, flow rate: 0.5 mL/min, gradient: 100 % B for 3 min, 100 - 75 % B in 14 min, 75 % B for 5 min and 100 % B for 11 min. Detection via HILIC-ESI-MS/MS.

Robustness. In comparison to RPLC, HILIC is known to be more sensitive to small changes of the chromatographic conditions [35]. It was investigated how even little modifications affect the chromatography.

In HILIC, the composition of the sample diluent can strongly impact the chromatography [25, 30, 36]. To test the robustness of the system regarding this parameter, the acetonitrile/Milli-Q ratio of the diluent was varied from 87.5/12.5 (v/v) to 92.5/7.5 (v/v). In general, the less retarded analytes were influenced most (Fig. 10), while the analytes with higher retention times were less impacted. Thus, for half of the compounds (Table A10) different levels of deterioration of the peak forms were observed, for three compounds it was even associated with a peak splitting. Frequently, the peak deteriorations were caused by an increase of the aqueous content of the diluent. These results highlight that a very good coherence of the injection solvent and the initial gradient composition has to be maintained to get symmetric Gaussian-like peak forms. The sensitivity to the diluent, a general rule in HILIC, was probably exacerbated by the use of high injection volumes (70 µL).

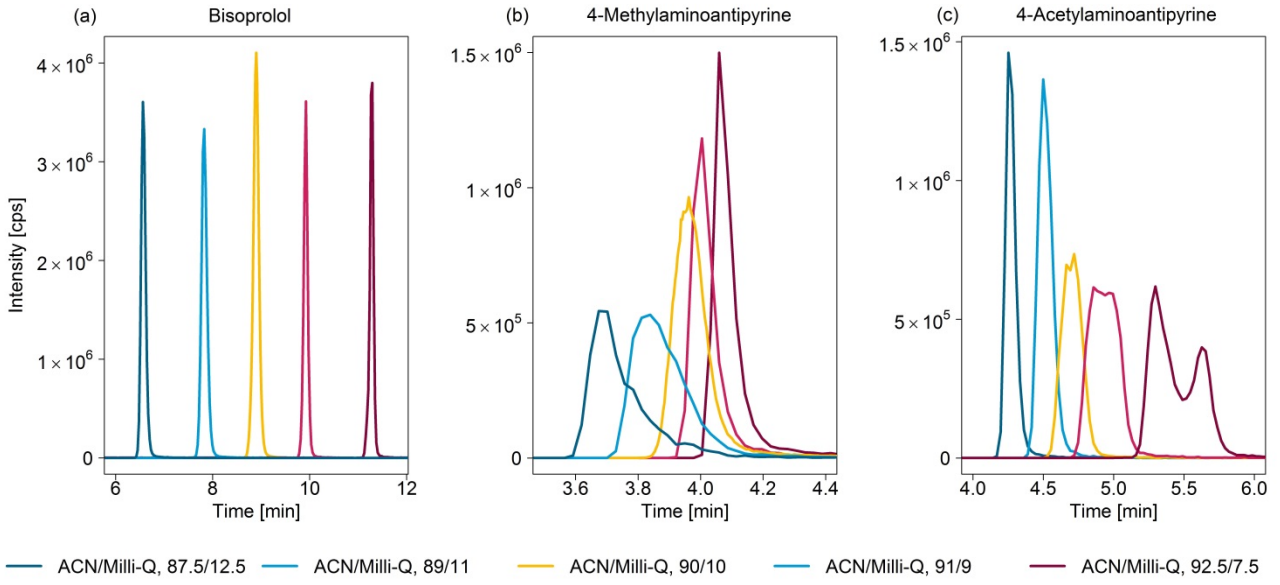
Figure 10: Retention times and peak forms of a 1000 ng/L multi-standard dissolved in different diluents. (Source: Own representation, Federal Institute of Hydrology)



(a) XIC of desmethylranitidine, (b) XIC of lamivudine, (c) XIC of gabapentin lactam, (d) XIC of 4-acetamidoantipyrine, (e) XIC of 4-methylaminoantipyrine. Conditions: column: HILIC Nucleodur (250 x 3 mm, 3 µm), eluent A: 10 mM ammonium formiate, 0.1 % formic acid, eluent B: acetonitrile/Milli Q, 90/10, v/v, 7.5 mM ammonium formiate, 0.1 % formic acid, flow rate: 0.5 mL/min, gradient: 100 % B for 3 min, 100 - 75 % B in 14 min, 75 % B for 5 min and 100 % B for 11 min. Detection via HILIC-ESI-MS/MS

Also slight variations of the acetonitrile/Milli-Q ratio of eluent B had significant effects on the retention times and the peak forms (Fig. 11). In contrast to the diluent, the change of the eluent composition affected all analytes, but to a different extent. For several compounds only the retention times were shifted, while for other analytes additionally the peak form was deteriorated or even split. For example, the beta blocker bisoprolol showed a dramatic shift of the retention time of 2.3 min, although the acetonitrile content was only increased by 2.8 % (Fig. 11).

Figure 11: Retention times and peak forms of a 1000 ng/L multi-standard measured with eluent B containing different ratio acetonitrile/Milli-Q. (Source: Own representation, Federal Institute of Hydrology)



(a) XIC of bisoprolol. (b) XIC of 4-acetamidoantipyrine. (c) XIC of 4-methylaminoantipyrine. Conditions: column: HILIC Nucleodur (250 x 3 mm, 3 µm), eluent A: 10 mM ammonium formiate, 0.1 % formic acid, eluent B: acetonitrile/Milli Q with different ratios, 7.5 mM ammonium formiate, 0.1 % formic acid, flow rate 0.5 mL/min, gradient: 100 % B for 3 min, 100 - 75 % B in 14 min, 75 % B for 5 min and 100 % B for 11 min. Detection via HILIC-ESI-MS/MS.

The influence of slight modifications of the ammonium formiate content in the composition of eluents A and B was also investigated. For both eluents, no modification of the chromatography was observed for small variations of the ammonium formiate content (up to 0.75 mM). As already indicated, the role of the buffer is assumed to attenuate the electrostatic interactions between the sulfobetaine moieties of the stationary phase and the analytes [37]. Since the chromatography was not impacted by an increase of the ammonium formiate concentrations, the buffer concentrations are obviously sufficiently high.

Application to environmental samples. The developed method was employed to determine the occurrence of the selected 27 analytes in WWTP effluents, surface water, groundwater and drinking water (Table 7). Detailed results are provided in the appendix (Table A11).

In WWTP effluents, 24 of 27 analytes were detected. Guanyl urea, the main transformation product of metformin [20], showed with 110 µg/L the highest maximum concentration. Metformin, the most prescribed pharmaceutical in Germany in 2015 [38], and its transformation product have shown to be very persistent in the aquatic environment [9]. The measured concentrations (0.71-4.2 µg/L for metformin and 3.6-110 µg/L for guanyl urea) are in accordance with those measured by Scheurer et al. (1.3-26 µg/L and 18-99 µg/L, respectively [9]). In addition to guanyl urea and metformin, six other compounds (4-formylaminoantipyrine, acesulfame, diatrizoate, gabapentin, gabapentin lactam and oxipurinol) reached median concentration of 1 µg/L and higher.

4-formylaminoantipyrine is one of the main metabolite of the analgesic drug metamizole [15]. Gabapentin is an antiepileptic pharmaceutical which has been analyzed in the µg/L range in WWTP effluent and surface water [39]. Oxipurinol is the main metabolite of allopurinol, an anti-gout drug [22]. Metamizole, gabapentin and allopurinol belong to the most frequently consumed pharmaceuticals in Germany (572, 79 and 134 t in 2015, respectively [38]). Diatrizoate is used at high doses (several mg/day) is only minimally metabolized and is persistent under aerobic conditions [40]. Acesulfame is widely used in food and beverages and is mainly excreted unmetabolized [41].

Only three analytes were not found in WWTP effluents, abacavir, N-acetyl-mesalazine and paracetamol. Abacavir is an antiviral drug which is subjected to quick degradation by photolysis [42] and biodegradation [17]. However, its main metabolite and biotransformation product, abacavir carboxylate [17, 43] was detected in WWTP effluents and also in groundwater at concentrations ranging from 11 ng/L to 170 ng/L. Paracetamol is known to be well degraded in WWTPs [44, 45], making its detection in the environment relatively rare in spite of its high consumption level (56 t in 2009 [46]). N-acetyl-mesalazine (N-acetyl-5-aminosalycilic acid) is the main metabolite of mesalazine (excreted from 8 to 77 % [21]) which belongs to the ten most prescribed pharmaceuticals in Germany (106 t in 2015 [38]). High removal rates of mesalazine in WWTPs have already been reported [47] and the non-detection of its metabolite let suppose a similar fate.

In surface water (Rhine, Saar, Horloff and Usa water), 19 of 27 analytes were found above LOQ. Oxipurinol and 4-formylamidoantipyrine showed the highest concentrations with 5.1 and 4.0 µg/L, respectively.

In groundwater, 19 of 27 analytes were identified. Acesulfame, diatrizoate, gabapentin and oxipurinol showed even concentrations above 1 µg/L. In drinking water, only the X-ray contrast medium diatrizoate and the artificial sweetener acesulfame were detected above LOQ, with 0.19 µg/L and 0.35 µg/L, respectively.

3.1.2.3 Conclusions

A multi-residue method was developed for extreme polar compounds in aqueous samples using HILIC comprising the determination of 11 pharmaceuticals, 15 metabolites and transformation products and acesulfame, used as an anthropogenic marker for treated wastewater. The selected polar pharmaceuticals cover a significant range of elevated polarity (log D at pH 7 ranged from –5.7 to 1.2), acidity (pKa ranged from 3.0 to 13.6) and basicity (pKb ranged from –0.8 to 12.3).

The study highlights that HILIC is extremely sensitive with regard to the acetonitrile/water ratio for both the eluent and the diluent. Thus, extreme care has to be taken that the eluent and the diluent composition are exactly adjusted and are not slightly changing over time, for instance due to a changing water contents in the solvents used. Hence, it is recommended to regularly replace all eluents of the mobile phases and to confirm that their composition is not changing.

Significant matrix effects could be attributed to the reduction of sodium adducts proportions by co-eluting anions such as nitrate or chloride. Thus, care has to be taken for analytes which are known to form adducts. If no labeled standard are available for quantification, either the co-eluting anions have to be removed before analysis or a matrix-matched calibration should be used.

Finally, it can be concluded that HILIC is appropriate to simultaneously quantify higher numbers of extreme polar organic compounds down to the low ng/L range in environmental samples from treated wastewater, surface water to groundwater and drinking water. However, method development is very complex and it is time consuming to find the optimum chromatographic conditions. Due to the low robustness compared to reversed phase methods, an extensive quality control is essential. Moreover, a very precise protocol and well trained lab personal companied with a frequent control of the chromatographic conditions are advisable to exclude incorrect results due to chromatographic problems. By reason of co-elution of the analytes with high concentrations of salts, the use of appropriate labeled internal standards is required for the analytes which tend to form adducts. However, these limitations are overmatched by the benefits of HILIC compared to other quantification methods. Even extreme polar compounds which show no retention on conventional stationary phases can be chromatographic separated and quantified at very low concentrations.

The study of environmental samples confirmed the presence of most of the selected extreme polar pharmaceuticals in the aqueous environment. The elevated concentrations measured for their metabolites and transformation products indicate their relevance for future monitoring campaigns.

Table 7: Concentration of the analytes detected in WWTP effluents, surface water, groundwater and drinking water.

	WWTP effluent (n=8)					Surface water (n=18)					Groundwater (n=15)					Drinking water
	Detection frequency [%]	Mean [µg/L]	Median [µg/L]	Maximal [µg/L]	Minimal [µg/L]	Detection frequency [%]	Mean [µg/L]	Median [µg/L]	Maximal [µg/L]	Minimal [µg/L]	Detection frequency [%]	Mean [µg/L]	Median [µg/L]	Maximal [µg/L]	Minimal [µg/L]	Concentration [µg/L]
4-Acetamidoantipyrine	100	1.5	0.96	5.5	0.29	94	0.28	0.17	0.9	<0.001	67	0.015	0.0072	0.063	<0.001	<0.001
4-Formylaminoantipyrine	100	9.2	9.1	11	7.6	89	0.51	0.24	4.0	<0.002	87	0.091	0.044	0.25	<0.001	<0.001
4-Methylaminoantipyrine	88	0.022	<0.02	0.055	<0.02	0	<0.005	<0.005	<0.02	<0.005	0	<0.001	<0.001	<0.001	<0.001	<0.001
9-Acridine carboxylic acid	100	0.18	0.17	0.28	0.098	83	0.048	0.03	0.32	<0.001	80	0.11	0.045	0.41	<0.001	<0.001
Abacavir	0	<0.01	<0.01	<0.01	<0.01	0	<0.005	<0.005	<0.01	<0.005	0	<0.001	<0.001	<0.001	<0.001	<0.001
Abacavir carboxylate	75	0.086	0.085	0.17	<0.02	0	<0.01	<0.01	<0.02	<0.01	7	<0.01	<0.01	0.011	<0.01	<0.001
Acesulfame	100	1.6	1.5	3.4	0.93	100	0.67	0.57	1.4	0.045	93	0.82	0.3	6.1	<0.001	0.350 ± 0.001
Acyclovir	87.5	0.11	0.091	0.25	<0.05	33	0.006	<0.002	0.070	<0.002	0	<0.001	<0.001	<0.001	<0.001	<0.001
Bisoprolol	100	0.3	0.3	0.41	0.19	89	0.03	0.016	0.20	<0.001	7	<0.001	<0.001	0.0026	<0.001	<0.0001
Clindamycin	100	0.086	0.091	0.13	0.046	89	0.042	0.018	0.18	<0.0005	47	0.0012	<0.0001	0.010	<0.0001	<0.0001
Clindamycin sulfoxide	100	0.28	0.27	0.39	0.20	89	0.039	0.046	0.12	<0.001	33	0.0012	<0.001	0.010	<0.001	<0.001
Diatrizoate	88	7.3	6.0	19	<0.05	89	0.67	0.69	1.8	<0.01	73	0.15	0.061	1.2	<0.01	0.190 ± 0.002
Emtricitabine	50	0.051	0.031	0.13	<0.005	28	0.0029	<0.001	0.045	<0.001	20	<0.001	<0.001	0.0039	<0.001	<0.001
Emtricitabine carboxylate	100	0.33	0.28	1.0	0.12	39	0.021	<0.01	0.11	<0.01	87	0.14	0.087	0.37	<0.005	<0.005
Emtricitabine *S*-oxide	50	<0.2	<0.2	0.38	<0.2	0	<0.05	<0.05	<0.2	<0.05	13	<0.01	<0.01	0.023	<0.01	<0.01
Gabapentin	100	3.9	3.7	7.3	2.8	89	0.93	0.67	3.3	<0.05	60	0.65	0.26	3.0	<0.05	<0.05
Gabapentin lactam	100	4.6	1.4	12	0.68	89	0.29	0.23	1.3	<0.01	60	0.036	0.016	0.14	<0.01	<0.01

	WWTP effluent (n=8)					Surface water (n=18)					Groundwater (n=15)					Drinking water
	Detection frequency [%]	Mean [µg/L]	Median [µg/L]	Maximal [µg/L]	Minimal [µg/L]	Detection frequency [%]	Mean [µg/L]	Median [µg/L]	Maximal [µg/L]	Minimal [µg/L]	Detection frequency [%]	Mean [µg/L]	Median [µg/L]	Maximal [µg/L]	Minimal [µg/L]	Concentration [µg/L]
Lamivudine	50	0.021	<0.02	0.058	<0.02	0	<0.005	<0.005	<0.02	<0.005	13	<0.001	<0.001	0.0018	<0.001	<0.001
Metformin	100	1.5	1.0	4.2	0.71	94	0.72	0.67	2.1	<0.005	40	0.037	<0.005	0.16	<0.005	<0.005
Guanyl urea	100	65	88	110	3.6	89	1.6	1.1	3.4	<0.02	7	<0.02	<0.02	0.032	<0.02	<0.02
N-acetyl mesalazine	0	<0.05	<0.05	<0.05	<0.05	0	<0.01	<0.01	<0.05	<0.01	0	<0.01	<0.01	<0.01	<0.01	<0.001
Oxipurinol	100	17	22	30	2.1	78	1.6	1.5	5.1	<0.2	67	0.62	0.21	1.8	<0.05	<0.05
Paracetamol	0	<0.25	<0.25	<0.25	<0.25	0	<0.02	<0.02	<0.25	<0.02	0	<0.005	<0.005	<0.005	<0.005	<0.005
Ranitidine	100	0.18	0.17	0.3	0.11	89	0.0056	0.0019	0.06	<0.0005	7	0.0003	<0.0001	0.0043	<0.0001	<0.0001
Desmethyl ranitidine	100	0.14	0.0091	1.1	0.0053	0	<0.005	<0.005	<0.005	<0.005	0	<0.005	<0.005	<0.005	<0.005	<0.005
Ranitidine *N*-oxide	100	0.018	0.021	0.037	0.0053	17	<0.005	<0.005	0.0040	<0.005	0	<0.005	<0.005	<0.005	<0.005	<0.005
Ranitidine *S*-oxide	100	0.029	0.03	0.038	0.02	61	0.0033	0.0025	0.0087	<0.001	0	<0.001	<0.001	<0.001	<0.001	<0.001

3.1.3 Middle polar pharmaceuticals

This method is for the rest of the high-priority pharmaceuticals. They are less polar and can be analyzed by reversed phase chromatography. There are already multimethods for similar analytes at the BfG [117-118]. These methods were used as starting conditions in method development. For the RPLC method, separation was performed using an Agilent Elipse Plus C18 column (150 x 2.1, 3.5 µm) equipped with a Zorbax SB-C8 column guard (2.1 x 12.5 mm).The flow rate was set to 300 µL/min. Eluent A was 0.1 % acetic acid and eluent B, acetonitrile. The following solvent gradient was applied: 0 to 1 min, 0 % B, 1 to 2 min 0 to 20 % B, 2 to 16 min, 20 to 100 % B, 16 to 19 min, 100 % B and 19 to 25 min, 0 to 100 % B. The injection volume was 80 µL and the column temperature was set to 25 °C. A list of the analytes and the substance specific parameters for mass spectrometric detection are shown in Table A12 and A13 in the appendix.

3.1.4 Hormone

Sensitive analytical methods are essential for environmental matrices as the steroidal hormones pose a threat on aquatic organism at very low concentrations down to the pg/L range [77]. Comprehensive analytical methods for the multi-residue determination of steroid hormones in environmental matrices are mainly missing. The published methods monitored a limited number of steroids, [78-80] focused on natural compounds [81-82] or investigated individual steroid hormone classes [83-87]. The aim of this study was to develop a robust, comprehensive and highly sensitive analytical method for the quantification of natural and anthropogenic steroids of different classes (PG, MC, GC) as well as their human metabolites in WWTP effluents and surface waters.

Table 8: hormonal pharmaceuticals

Glucocorticoids (GC)		Glucocorticoids (GC)	
Abbr.	**Substance**	**Abbr.**	**Substance**
BEC	Beclomethasone	DFCval	Diflucortolone 21-valerate
BECprop	Beclomethasone 17-propionate	FMS	Flumethasone
BECdiprop	Beclomethasone 17,21-dipropionate	FMSpiv	Flumethasone 21-pivalate
BMS	Betamethasone	FCNact	Fluocinolone acetonide
BMSac	Betamethasone 21-acetat	FML	Fluorometholone
BMSval	Betamethasone 17-valerat	FLUfur	Fluticasone 17-furoate
BMSprop	Betamethasone 17-propionat	FLUprop	Fluticasone 17-propionate
BMSdiprop	Betamethasone 17,21-dipropionate	HAL	Halcinonide
BDN	Budesonide	HLM	Halometasone
BDN-m1	6ß-Hydroxy budesonide	MPNL	Methylprednisolone
CIC	Ciclesonide	MPNLacp	Methylprednisolone 21-acetate 17-propionate
CIC-m1	Desisobutyryl ciclesonide	MPNLprop	Methylprednisolone 21-propionate
CLO	Clobetasol	MOM	Mometasone
CLOprop	Clobetasol 17-propionate	MOMfur	Mometasone 17-furoate
HCOR	Cortisol (Hydrocortisone)	PNL	Prednisolone
COR	Cortisone	PNS	Prednisone
DMS	Dexamethasone	TRIact	Triamcinolone acetonide
DMS-m1	6ß-Hydroxy dexamethasone	TRIact-m1	6ß-Hydroxy triamcinolone acetonide
DMSac	Dexamethasone 21-acetate		
Progestogens (PG)		**Mineralocorticoids (MC)**	
Abbr.	**Substance**	**Abbr.**	**Substance**
CLM	Chlormadinone	CAN	Canrenone
CLMac	Chlormadinone acetate	CAN-m1	11α-Hydroxy canrenone
CYP	Cyproterone	FLC	Fludrocortisone
CYPac	Cyproterone acetate	FLCac	Fludrocortisone 21-acetate
DIE	Dienogest	SPL	Spironolactone
DIE-m1	6ß-Hydroxy dienogest	SPL-m1	7α-Thiomethyl spironolactone
DPN	Drospirenone		
ETG	Etonogestrel		
GES	Gestodene		
HPG	17α-Hydroxy progesterone		
LNG	Levonorgestrel		
MPR	Medroxy progesterone		
MPRac	Medroxy progesterone acetate		
MPRac-m1	6ß-Hydroxy medroxy progesterone acetate		
MEG	Megestrol		
MEGac	Megestrol acetate		
NES	Norethisterone		
NESac	Norethisterone acetate		

3.1.4.1 Materials and Methods

Reagents and Materials. HPLC grade methanol and n-hexane were obtained from Sigma-Aldrich (Seelze, Germany) and Pico grade acetone was purchased from Promochem® (LGC Standards, Wesel, Germany). Milli-Q water was obtained from Millipore (18.2 MΩ, Merck, Darmstadt, Germany). Reference standards and isotope-labeled substances (Tab. A14) were all purchased from Sigma-Aldrich, Santa Cruz Biochemical (Dallas, USA) or Toronto Research Chemicals Inc. (Ontario, Canada).

Sampling of Wastewater Effluents and Surface Water. Treated wastewater was collected from five conventional municipal German WWTPs. The sample locations of river and surface water were chosen in the instance to get a broad spectrum of river types. All samples were taken as grab samples either from the effluent discharge or below the water surface close to the river bank. Sampling date, WWTP capacities and locations are shown in Table A14 and Figure A2. In addition, river water was collected upstream and downstream of three WWTPs discharges.

Target Compound Selection. The synthetic steroid hormones were selected based on i) the application quantity prescribed in Germany in 2014 [90] (number of prescribed daily dose x defined daily dose) and, ii) in case of glucocorticoids on their relative potencies according to ATC-codes48 (Anatomical Therapeutic Chemical). The steroid hormone types progestogens (PG), glucocorticoids (GC), mineralocorticoids (MC) and some of their main commercially available metabolites were included in the developed method. Androgens were not considered due to their limited use in medicinal therapy. In total, 60 analytes comprising 18 PG, 37 GC and 5 MC were integrated into one analytical method. Application quantities of the selected compounds, and further information regarding analytes are shown in Table A16. Optimized MS parameters of the target analytes are summarized in Table A14.

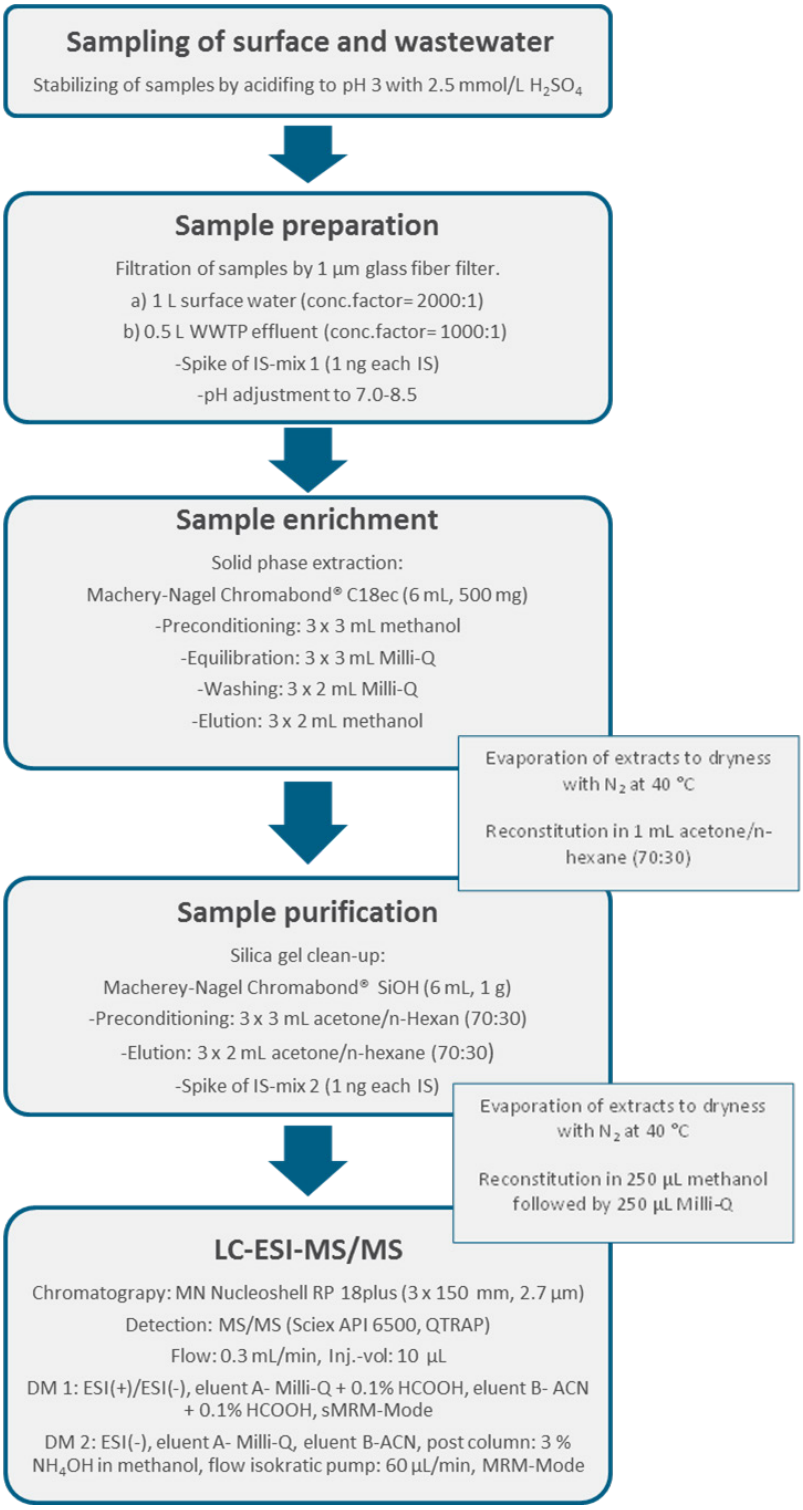
Sample Pretreatment. All samples were collected in cleaned and baked (at 550 °C for 8 h) glass bottles. If samples could not be extracted within 24 h, acidification to pH 3 with sulfuric acid was performed to prevent biodegradation. The water samples were cooled down to 4 °C during transport to the laboratory and afterwards filtered using a 1 µm glass fiber filter (Whatman, GF6, Maidstone, United Kingdom). The filtered samples were finally adjusted to pH 7-8.5 with diluted ammonia solution or sulfuric acid prior to enrichment.

Solid Phase Extraction (SPE). For sample enrichment, 500 mL filtered WWTP effluent and 1000 mL surface water were spiked with 1 ng of each surrogate standard from IS-mix 1 prior to SPE. The water samples were loaded onto end-capped C18 cartridges (C18ec, 6 mL, 500 mg, Macherey-Nagel, Düren, Germany), which were preconditioned with 3 x 2 mL methanol followed by 3 x 3 mL Milli-Q. Water samples were passed through the cartridges by gravity within 12 h. The cartridges were rinsed with 3 x 2 mL Milli-Q and dried by nitrogen for approximately 2 h. For elution of the extracted analytes 3 x 3 mL methanol was used. Subsequently, the extracts were evaporated to dryness under a gentle stream of nitrogen at 40 °C and were re-dissolved with 300 µL n-hexane and 700 µL acetone for further clean up. If the cartridges were not eluted immediately, they were stored at -20 °C in the dark after drying. The schematic workflow of the developed method is shown in Figure 11.

Sample Clean Up. Purification was achieved by commercially available silica gel glass cartridges (1 g, 6 mL, Macherey-Nagel). The silica gel was dried for 2 h at 100 °C prior to usage. Polarity and composition of the elution solvent was optimized for the target analytes. The cartridges were preconditioned with 3 x 3 mL n-hexane/acetone (3:7). Afterwards, the sample extracts were loaded onto the cartridges and were eluted three times with 2 mL n-hexane/acetone (3:7). Since, several esterified internal standards (e.g. betamethasone dipropionate-d10, betamethasone propionate-d5) hydrolyzed during the sample treatment, we spiked 4 deuterated internal standards after the sample clean-up (IS-mix 2) to prevent the hydrolysis of these surrogates. Otherwise, it would lead to contaminations of the samples with non-labeled steroids. Therefore, 1 ng of each surrogate standard from IS-mix 2 was spiked to

the extracts after clean-up. Then, the extracts were evaporated under a gentle stream of nitrogen at 40 °C to dryness and reconstituted with 250 µL methanol and 250 µL Milli-Q for LC-MS/MS analysis.

Figure 12: Workflow of the developed method for the trace quantification of 60 steroid hormones by LC-MS/MS. (Source: Own representation, Federal Institute of Hydrology)



High-Performance Liquid Chromatography and Tandem Mass Spectrometry. The analysis was performed with an HPLC system, consisting of a G1367E autosampler, a G1330B cooling thermostat for the autosampler, a G1312B binary HPLC pump, a G1310B isocratic HPLC pump, a G1379B membrane degasser and a G1316A column oven (all Agilent 1260Infinity Series, Waldbronn, Germany). Separation was achieved with a MN Nucleoshell RP 18plus column (3 x 150 mm, 2.7 µm) (Macherey-Nagel) with a flow rate of 0.3 mL/min. The injection volume was 10 µL and column oven temperature was set to 25 °C. Sensitive quantification was achieved by splitting LC-MS/MS analysis in different chromatographic runs. Milli-Q with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B) were used as mobile phases for detection-method 1 (DM 1, ESI[+/-]). Detection-method 2 (DM 2, ESI[-]) operates with non-acidified eluents Milli-Q (C) and acetonitrile (D). To increase the ionization efficiency of the targeted analytes in DM 2, a post column addition of a 3% ammonia solution in methanol with a flow rate of 0.06 mL/min was applied by using an isocratic pump and a mixing tee according to previous literature [102-103].

In order to avoid a co-elution of interfering substances, the LC gradient for both detection methods was optimized as follows: from 0 to 0.5 min 10% B or D; from 0.5 min to 15 min gradual increase to 47% B or D; then B or D was linearly increased up to 98% in 5 min and held for 10 min; finally returning to 10% B or D in 0.1 min and held for 5 min for equilibration at the end of each chromatographic run, in total 35 min. The HPLC system was coupled to a triple-quadrupole mass spectrometer system (QqQ-LIT-MS, API 6500 QTrap, Sciex, Darmstadt, Germany) equipped with an IonDrive™ ion source for electrospray ionization (ESI). The general MS parameters for both detection-methods were: ion source gas 1 (GS1) and ion source gas 2 (GS2) 35 psi; curtain gas (CUR) 45 psi; collision gas (CAD) medium; source temperature (TEM) 400 °C; ion spray voltage for negative and positive ionization mode -4500 V/5500 V; entrance potential (EP) -10 V/10 V; collision cell exit potential (CXP) -14 V/ 14 V.

DM 1 was performed with switching polarities within the chromatographic runs using scheduled multiple reaction monitoring (sMRM) mode. The specific parameters in DM 1 were as follows: MRM detection window 50 s; target scan time 0.6 s and settling time 4 ms.

DM 2 operates only in negative ionization mode using multiple reaction monitoring (MRM) with adjusted dwell times of 20 ms for all MRM transitions.

MS data acquisition was controlled with Analyst 1.6.3 (Sciex). For identification and quantification, the two most sensitive MRM transitions of each analyte were monitored (Tab. A14).

Quantification and Quality Control. An external calibration in the concentration range 0.005–50 ng/mL was used for quantification. Linear regression was applied to the calibration curves with a weighting factor 1/x. The peak areas of the analytes were corrected by one of the 18 isotope-labeled surrogates. Furthermore, a random control standard was measured every tenth sample within a sequence for quality control. Finally, data were processed with the software MultiQuant 3.0.2 (Sciex).

Method Validation. In order to validate the developed method, recoveries and repeatability were examined over the complete concentration range for river water and WWTP effluent. All samples were processed in quadruplicate. Surface water was spiked with 0.05 ng/L, 0.25 ng/L, 0.5 ng/L and 5 ng/L of each analyte, while WWTP effluent was spiked with concentrations of 0.5 ng/L, 1 ng/L, 10 ng/L and 50 ng/L. Due to partial hydrolysis of several glucocorticoid esters, the validation of betamethasone, dexamethasone, beclomethasone and methylprednisolone was conducted in surface water at two concentration levels (0.5 ng/L and 5 ng/L) in separate experiments, for their i) diesters, ii) monoesters and iii) free alcohols. For the determination of the limit of detection (LOD) and limit of quantification (LOQ), the Software PeakView® 2.2.0 (Sciex) was used. By definition, the calculations were based on a

signal-to-noise (SN) ratio of 3 (LOD) and 10 (LOQ) either using the background concentration or a total spike amount in the smoothed (smoothing factor: 2.0) chromatograms of environmental samples. Noise area was selected manually from the background that bordered on the chromatographic peak. For determination of LOD and LOQ, the 3σ SN values were used and extrapolated accordingly.

3.1.4.2 Results and Discussion

Method Performance. The calibration curves of all analytes showed a good linearity (R >0.99) in the defined concentration range. The peak widths were approximately 0.3 min for all analytes. LC gradient was optimized to separate the interfering analyte pairs with similar or even identical molar masses. Most synthetic steroids consist of the similar steroid structure and the same functional groups. Thus, for quantification it is essential to achieve an appreciable chromatographic separation (i.e. for epimers beta- and dexamethasone or cortisone/prednisolone). The developed chromatographic method showed no interfering substances and all critical steroid pairs were base-line separated.

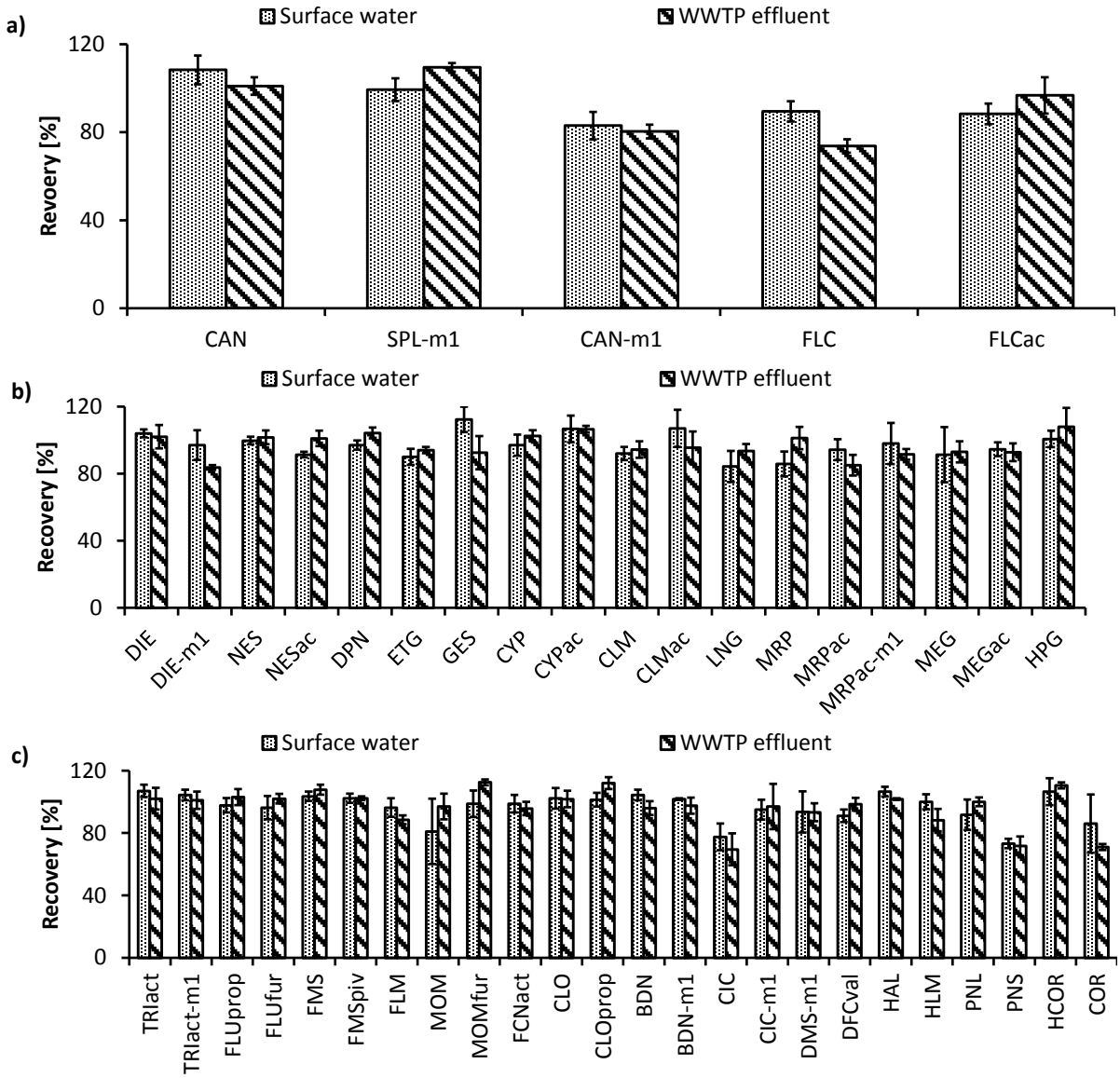
In recent studies [83, 86] spironolactone was monitored using as precursor the in-source fragment m/z=341. As canrenone forms the same precursor and fragments, an insufficient chromatographic separation of canrenone and spironolactone lead to incorrect evaluations. Unfortunately, the separation of these two compounds needs a slowly increasing gradient leading to very long retention times and expanded peak widths with the column used (RP C18ec) [104]. Hence, we decided to exclude spironolactone from the target analytes and exclusively monitoring its qualifier ion (417 → 341). However, spironolactone was not detected in any water sample.

Moreover, to achieve low LOQs we compared the detection sensitivities of formiate adducts $[M+HCOO]^-$ with those of $[M+H]^+$ ions in surface waters with acidified eluents (detection method 1), since GC and MC preferentially form carboxylic adducts (formiate and acetate) in ESI. It was already reported that the analysis of these adducts might increase the sensitivity of detection for steroids [83-84, 87]. However, several steroids (e.g. flumethasone pivalate, halcinonide) showed low LOQs when $[M-H]^-$ ions were considered for fragmentation in non-buffered eluents and addition of ammonia solution after the chromatography (detection method 2) according to Gentili et al.[102] and Schlüsener et al. [103].Finally, the analytical method was split in to two chromatographic runs, to reach low LOQs. For 12 steroids the $[M+HCOO]^-$ adduct ions were used for quantification (solely un-esterified GC). For 39 analytes a higher sensitivity was observed when using $[M+H]^+$ -ions and for 9 steroids most suitable results were achieved when $[M-H]^-$-ions were used for the fragmentation in detection method 2.

For further increase of sensitivity a silica gel clean-up was used after sample extraction, to remove matrix impurities as described in previous studies [62, 79, 83] By these improvements, LOQs in the range of 0.02 ng/L (e.g. cortisone) to 0.5 ng/L (e.g. drospirenone) in surface water and from 0.05 ng/L (e.g. betamethasone) to 5 ng/L (chlormadinone) in treated wastewater could be achieved (Tab. A17). To the best of our knowledge, this is the first reported comprehensive analytic method for the simultaneous determination of 60 multi class steroids in environmental waters down to the pg/L range.

Method Validation. As shown in Figure 13, relative recoveries ranged from 73 ± 3% (prednisone) to 112 ± 8% (gestodene) in river water and from 70 ± 10% (cortisone) to 113 ± 2% (mometasone furoate) in WWTP effluents (for recoveries in detail, see Table A18). The recoveries of the analytes were similar at all spiked concentrations and showed no significant scattering or trends. Moreover, the results were comparable for all steroid types as well as for river water and treated wastewater in the considered concentration range.

Figure 13: Recoveries (Source: Own representation, Federal Institute of Hydrology)

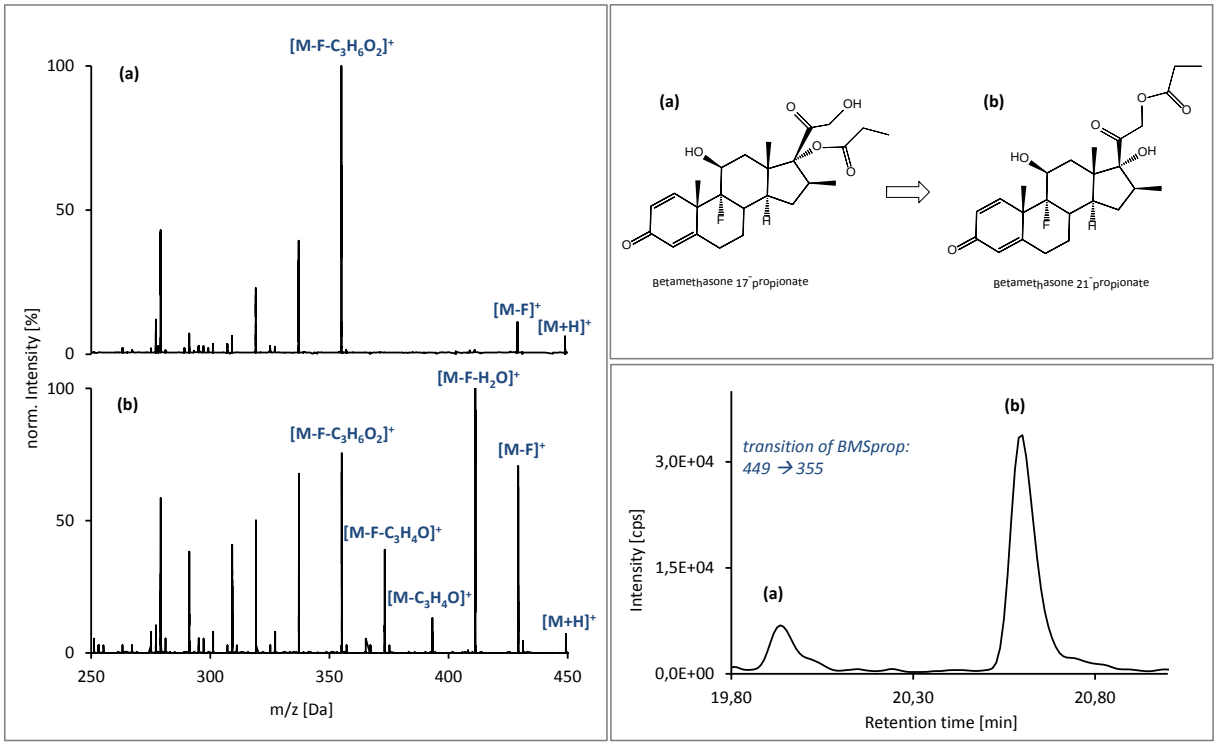


Recoveries (corrected by isotope-labeled surrogates) of a) mineralocorticoids, b) progestogens and c) glucocorticoids in surface water and WWTP effluents. Recovery rates were averaged over the 4 concentration levels. The error bars express the relative standard deviation (RSD%).

For validation of the analysis of glucocorticoidal diesters of betamethasone, beclomethasone and 6α-methylprednisolone we chose a different approach for the determination of the recoveries, since these diesters are known to hydrolyze rapidly to their active monoester metabolites. Moreover, the spontaneous isomerization of these C17-monoesters to the C21-esters as well as a continuing ester cleavage is already known from several pharmacokinetic studies [105-107] This phenomenon of the isomerization is described in the literature as acyl-migration[107] and was observed at neutral pH (8.14 [106] and 7.4 [107]) for several glucocorticoid monoesters. Thus, these rearrangements are likely to occur in the aquatic environment. C17-monoesters of further target compounds did not show any isomerization, due to structural barriers. For instance, the substitution of the C21-hydroxyl group with chlorine as present in clobetasol propionate and mometasone furoate hinders an isomerization leading to more stable esterified GCs [108]. As a consequence of the acyl-migration, two chromatographically separated peaks were detected for both transitions of betamethasone propionate, betamethasone valerate,

beclomethasone propionate and 6α-methylprednisolone propionate which were confirmed by high-resolution mass spectrometry and finally quantified as the sum of both peaks (C17/C21-monoester) as shown exemplarily for betamethasone propionate in Figure 14. Differences in the MS^2-spectra of both esters could be attributed to the secondary hydroxyl group at position C17 in the C21 monoester, which leading to a loss of H_2O in the fragmentation. Furthermore, to compare the sensitivity of the isomeric monoesters, we determined the sum of peak areas in water samples that were spiked at different sample preparation steps, since the ratios of C17/C21-monoesters differ depending to their dwell times in aqueous media. The summed peak areas of C17/C21-esters were almost constant, regardless of the extent of migration, thus their detection sensitivities were comparable. It should be noted that the corresponding deuterated internal standards (e.g. betamethasone dipropionate-d10, betamethasone propionate-d5) hydrolyzed in the same way during the sample treatment. As the hydrolysis of the deuterated standards leads to contamination with non-labeled steroids, we spiked this group of deuterated internal standards after the sample clean-up (IS-mix 2). The esters were stable in the methanolic standards as well as in the final diluent (methanol/Milli-Q 1:1).

Figure 14: Chemical structures, extracted ion chromatogram of non-spiked WWTP effluent and high-resolution MS^2-spectra. (Source: Own representation, Federal Institute of Hydrology)



(a) betamethasone 17-propionate (accurate mass= 449.2655 Da) and (b) betamethasone 21-propionate (accurate mass= 449.2423 Da). High-resolution MS^2-spectra was recorded with TT6600 (Sciex) at same conditions as adjusted for the target method (CE= 20 eV, Cone voltage= 5500 V)

The recovery rates and reproducibility of analysis of i) diesters, ii) monoesters and iii) steroid alcohols were determined separately for surface water at two different concentrations (0.5 ng/L and 5.0 ng/L). Recoveries were calculated as the sum of the spiked compound and its formed hydrolysis products. As shown in Table 9, this validation approach revealed reproducible and almost closed recoveries in all experiments. Therefore, we were able to verify that all target steroids and metabolites were quantitatively recovered. Total recoveries of the diester ranged from 90 ± 9% (BECdiprop) to 108 ± 6%

(BMSdiprop) and for the steroid alcohols from 86 ± 2% (BEC) to 110 ± 7% (BMS). The monoesters of betamethasone, dexamethasone and 6α-methylprednisolone revealed good recoveries close to 100%. Lower recoveries of beclomethasone propionate might be caused by different sensitivities for quantification of C17 and C21 propionate.

Table 9: Steroid ester decomposition during the sample treatment. Total recoveries of the formed analytes. Errors representing the reproducibility (expressed as the 95%-confidence intervals) of targeted glucocorticoidal i) diester, ii) monoester and iii) alcohols. Monoesters of betamethasone (BMS) were evaluated in sum, thus their summed recovery was divided appropriate (by 3).

	Recovery [%], c=0.5 ng/L					Recovery [%], c=5.0 ng/L				
Substance	**n1**	**n2**	**n3**	**n4**	**Mean ± 95%-CI**	**n1**	**n2**	**n3**	**n4**	**Mean ± 95%-CI**
i) diesters										
BMSdiprop	87	91	82	88	87 ± 6	93	88	93	93	92 ± 4
BMSprop	12	11	14	13	12 ± 2	6	8	8	14	9 ± 6
BMS	11	5	13	10	9 ± 6	<1	1	<1	2	<1
Σ	**111**	**107**	**109**	**110**	**108 ± 6**	**99**	**97**	**101**	**109**	**102 ± 8**
MPNLacp	69	72	62	68	68 ± 7	80	73	73	66	73 ± 9
MPNLprop	37	27	31	38	33 ± 8	26	29	30	47	33 ± 15
MPNL	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Σ	**105**	**99**	**93**	**106**	**101 ± 10**	**105**	**102**	**103**	**113**	**106 ± 8**
BECdiprop	94	89	82	86	88 ± 9	94	88	89	93	91 ± 5
BECprop	4	<1	2	4	3 ± 3	2	3	3	5	3 ± 2
BEC	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Σ	**98**	**89**	**84**	**89**	**90 ± 9**	**96**	**91**	**92**	**98**	**94 ± 5**
ii) monoesters										
BMSac	92	105	84	119	100 ± 24	105	94	101	98	99 ± 7
BMSprop	82	87	80	81	82 ± 5	94	91	97	93	94 ± 4
BMSval	63	71	62	86	71 ± 18	69	62	63	65	65 ± 4
BMS	18	32	21	24	24 ± 10	21	14	13	12	15 ± 6
Averaged Σ	**85**	**98**	**83**	**103**	**95 ± 17**	**96**	**87**	**91**	**89**	**91 ± 6**
MPNLprop	80	80	85	80	81 ± 4	81	79	83	83	82 ± 3
MPNL	18	21	17	22	19 ± 4	22	16	16	15	17 ± 5
Σ	**98**	**101**	**102**	**102**	**101 ± 3**	**104**	**95**	**99**	**97**	**99 ± 6**
BECprop	37	46	41	63	47 ± 18	44	41	42	44	43 ± 3
BEC	8	12	10	11	10 ± 2	12	9	9	8	10 ± 2
Σ	**45**	**57**	**50**	**74**	**57 ± 20**	**56**	**50**	**51**	**53**	**52 ± 4**
DMSac	89	98	83	92	91 ± 13	95	93	96	93	94 ± 4
DMS	11	16	13	10	13 ± 6	13	13	13	12	13 ± 1
Σ	**100**	**114**	**97**	**102**	**103 ± 17**	**107**	**106**	**109**	**105**	**107 ± 4**

Substance	Recovery [%], c=0.5 ng/L					Recovery [%], c=5.0 ng/L				
	n1	n2	n3	n4	Mean ± 95%-CI	n1	n2	n3	n4	Mean ± 95%-CI
iii) alcohols										
BMS	96	109	113	100	**105 ± 13**	104	113	111	114	**110 ± 7**
MPNL	89	99	96	93	**94 ± 7**	95	93	97	99	**96 ± 4**
BEC	86	86	87	84	**86 ± 2**	98	94	100	103	**98 ± 6**
DMS	98	96	101	91	**97 ± 7**	101	106	104	108	**105 ± 5**

Occurrence of Steroid Hormones in Environmental Samples

Mineralocorticoids (MC). The developed analytical method was applied to several effluents from municipal WWTPs and various rivers and streams to monitor the discharge and occurrence of different types of steroidal pollutants. Concentrations of the most frequently detected analytes are summarized in Table 10. Among MC, the spironolactone metabolites canrenone and 7α-thiomethyl spironolactone were commonly present in WWTP effluents, rivers and streams. Measured concentrations of canrenone ranged up to 19 ng/L in WWTP effluents and up to 8.3 ng/L in the rivers and streams containing an elevated percentage of treated wastewater. The concentrations of 7α-thiomethyl spironolactone were lower, ranging up to 2.3 ng/L in WWTP effluents and up to 1.3 ng/L in surface waters. Both metabolites were found to be ubiquitously present in nearly all analyzed water samples and hence should be considered in further monitoring campaigns of steroid hormones. In addition, 11α-hydroxy canrenone was detected in WWTP effluent 1 and in the receiving surface water SW-1b (Tab. A18). In contrast to its metabolites, spironolactone was not detected at all, because spironolactone is rapidly metabolized in humans to canrenone, 7α-thiomethyl spironolactone as well as to other metabolites [108]. In Germany its annual consumption accounts for 9.2 t in 2014. The instability of spironolactone in contact with activated sludge and in aqueous solutions was shown elsewhere [109]. In spite of the high metabolism and fast degradation, the environmental relevance of spironolactone and its major metabolite canrenone has been revealed, after abnormal fish were spotted in the vicinity of a chemical plant producing the steroidal compounds [70-71]. Chemical analysis confirmed the high concentrations of both pollutants (spironolactone, canrenone) in the river water downstream of a pharmaceutical manufacturer [90]. In addition, La Lone et al. [65] observed antiandrogenic effects on fish which were exposed to spironolactone. Therefore, spironolactone and its active metabolite canrenone pose a potential risk to biota.

Table 10: Occurrence of most commonly detected steroid hormones in various municipal WWTP effluents and surface waters in Germany. (< = below detection limit, <LOQ= above detection limit, below quantification limit)

	Wastewater treatment plant effluent Concentration [ng/L]						Surface water Concentration [ng/L]											
	1	2	3	4	5	LOD/LOQ	Mühlen-bach (down-stream WWTP 1), SW-1b	River Nahe (down-stream WWTP 2), SW-2b	Schwelme (down-stream WWTP 3), SW-3b	River Wupper (down-stream Schwelme), SW-4b	Tel-tow ca-nal, SW-5	Landgra-ben (down-stream WWTP), SW-6	River Neckar SW-7	Riv-er Main, SW-8	River Lahn,SW-9a	River RhineSW-10d	River Ahr, SW-11	LOD/LOQ
Mineralocorticoids (MC)																		
Canrenone	4.5	3.7	10	19	8.0	0.4/ 1.4	3.0	1.6	8.3	1.2	2.9	1.8	0.6	0.4	0.8	0.5	0.2	0.08/0.2
7α-Thiomethyl spironolactone	0.2	1.2	1.5	3.8	2.0	0.05/ 0.2	0.1	0.3	1.3	0.2	0.6	0.2	0.07	0.08	0.3	0.05	<LOQ	0.01/0.03
Glucocorticoids (GC)																		
6β-Hydroxy triamcinolone acetonide	1.2	1.7	6.9	2.3	2.2	0.06/ 0.2	0.9	0.2	5.1	0.6	1.2	0.8	<	0.1	0.08	0.05	<	0.03/0.05
Betamethasone	0.6	0.4	0.05	0.2	0.6	0.02/ 0.05	0.5	0.2	0.4	0.04	1.0	0.3	0.1	0.1	0.1	<	<LOQ	0.02/ 0.05
Betamethasone propionate	1.1	1.5	1.2	3.6	0.3	0.08/ 0.2	0.9	0.2	0.6	0.07	0.4	1.2	<LOQ	<	0.07	0.09	<	0.02/ 0.05
Betamethasone valerate	1.3	2.5	1.1	2.2	1.2	0.08/ 0.3	0.9	0.2	0.7	<LOQ	0.2	1.3	<	<LOQ	<LOQ	<LOQ	<	0.03/ 0.2
Budesonide	<	<	1.2	2.0	<	0.5/ 1.0	<	<	0.7	<LOQ	<	<LOQ	<	<	<	<	<	0.2/ 0.5
Sum of BMS derivatives	*3.0*	*4.4*	*2.4*	*6.0*	*2.1*		*2.3*	*0.6*	*1.7*	*0.1*	*1.6*	*2.8*	*0.1*	*0.1*	*0.17*	*0.09*	*<LOQ*	
Clobetasol propionate	0.5	0.8	2.1	4.0	5.4	0.08/ 0.3	0.4	0.2	3.4	0.3	1.7	0.2	0.05	0.1	0.1	0.06	<	0.02/ 0.05
Cortisol	0.9	1.4	1.2	2.8	0.9	0.06/ 0.2	0.7	1.3	1.3	0.4	0.2	1.0	0.6	0.7	1.3	0.3	0.7	0.02/ 0.08
Cortisone	0.2	0.3	0.4	0.9	0.2	0.1/ 0.2	0.2	0.4	0.7	0.3	0.08	0.2	0.6	0.7	1.0	0.1	0.2	0.01/ 0.02

Fluocinolone acetonide	0.1	0.1	0.1	0.2	0.2	0.03/ 0.1	0.09	<LOQ	0.1	<LOQ	0.09	0.1	<	<	<LOQ	<	<	0.02/ 0.05
Fluticasone propionate	<LOQ	0.1	0.5	1.0	0.9	0.05/ 0.1	<LOQ	<LOQ	0.4	0.06	0.3	0.2	<LOQ	<	<LOQ	<	<	0.05/0.10
Methylpredniso-lone	<LOQ	<	0.1	1.0	0.2	0.02/ 0.06	<LOQ	<LOQ	0.2	0.05	0.2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<	0.01/ 0.05
Methylpredniso-lone propionate	1.4	<LOQ	2.4	0.5	4.2	0.2/ 0.5	0.9	<	1.3	<LOQ	0.9	0.6	<	<	<	<	<	0.06/ 0.2
Sum of MPNL derivatives	*1.4*	*<0.5*	*2.5*	*1.5*	*4.4*		*0.9*	*<LOQ*	*1.5*	*0.05*	*1.1*	*0.6*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	<	
Mometasone furoate	0.8	1.2	1.7	2.2	1.4	0.08/ 0.3	0.6	<LOQ	1.0	<LOQ	0.2	0.8	<	<	<LOQ	<	<	0.05/ 0.2
Prednisolone	<LOQ	<LOQ	0.3	0.6	<LOQ	0.06/ 0.2	0.05	0.07	0.4	0.06	<LOQ	0.05	0.1	0.07	0.05	0.08	0.05	0.02/ 0.05
Prednisone	<LOQ	<LOQ	0.2	0.4	<LOQ	0.06/ 0.2	<LOQ	<LOQ	<LOQ	<LOQ	<	0.05	<	<LOQ	<LOQ	<LOQ	<	0.03/ 0.05
Triamcinolone acetonide	6.3	5.5	17	11	28	0.1/ 0.5	4.4	1.0	12	1.5	7.6	8.5	0.3	0.6	0.3	0.3	0.05	0.01/0.04
Progestogens (PG)																		
Dienogest	3.3	1.3	4.4	4.3	1.4	0.2/ 0.3	2.3	0.2	2.0	0.3	<	0.1	0.05	0.05	0.09	<LOQ	<	0.02/ 0.05
6ß-Hydroxy dienogest	<LOQ	0.6	0.6	0.6	0.9	0.2/ 0.4	<	<	0.4	<	<	0.5	<	<	<	<	<	0.05/ 0.1
Cyproterone acetate	0.8	1.7	2.9	3.7	2.3	0.3/ 0.8	0.6	0.2	2.6	0.3	0.9	0.6	<	<	<	<	<	0.05/ 0.2
17α-Hydroxy progesterone	1.1	0.7	0.7	1.0	1.3	0.3/ 0.7	0.6	<LOQ	<LOQ	<	<	0.6	<	<	<	<	<	0.25/ 0.5

Glucocorticoids (GC). Due to the structure diversity of synthetic GCs used and the wide range of medicinal applications, this hormone class is the largest group of target hormones in this study. Concentrations of the most frequently detected GCs are summarized in Table 9. Further results of all analytes and samples are shown in Table A17.

In total, 23 of 37 GCs were found in at least one sample and 14 of them were present in all five WWTP effluents above the LODs. Triamcinolone acetonide and its metabolite 6β-hydroxy triamcinolone acetonide were found as the predominant GC compounds in our sampling campaign, since they accounted for 39-66% of the total GC concentration in the WWTP effluents. The concentrations of triamcinolone acetonide ranged from 5.5 ng/L to 28 ng/L in WWTP effluents and its metabolite was found with concentrations between 1.2 ng/L and 6.9 ng/L, respectively. Furthermore, the measured concentrations of triamcinolone acetonide are in good agreement with studies analyzing a Dutch WWTP effluent (14 ng/L)[91] and WWTP effluents in the U.S. (6-14 ng/L) [83]. Triamcinolone acetonide was detected in our study in 20 of 22 river and stream samples above the LOQ (0.04 ng/L) ranging from 0.04 ng/L to 12 ng/L. This indicates the ubiquitous presence of triamcinolone acetonide in rivers and even streams with a relative low percentage of treated wastewater. Furthermore, triamcinolone acetonide as well as its bi-fluorinated analogue fluocinolone acetonide were reported to be relatively stable during laboratory degradation experiments with activated sludge [92]. Although, concentrations of fluocinolone acetonide were in all cases below 1 ng/L due to low consumption in Germany (12 kg in 2014).

Residues of mometasone furoate and fluticasone propionate have been detected in all WWTP effluents at concentrations up to 2.2 ng/L and 1.0 ng/L, respectively. The detection frequency of fluticasone propionate was comparable to WWTP effluents in the U.S..30 However, mometasone furoate was detected for the first time above LOQ in treated wastewaters and in 4 rivers and streams (0.2 - 1.0 ng/L). Although, it is ranked in the top 100 consumed pharmaceuticals in the United States, no residues were detected by Jia et al. [83]. This difference might be caused by our lower detection limit (0.08 ng/L in effluent) compared with the literature (5 ng/L in effluent) [83]. It should be noted that in Germany fluticasone propionate and mometasone furoate are over-the-counter drugs for the treatment of seasonal rhinitis. Therefore, the totally used quantities might be higher than calculated, caused by their additional usage in non-prescription products, thus their discharge into water bodies may vary from season to season.

Moreover, traces of further GC were detected less frequently such as beclomethasone (0.07 ng/L) and flumethasone pivalate (0.05 ng/L) above LOQ in several surface waters (Tab. A17).

The natural steroids cortisone and cortisol were detected in all water samples. In WWTP effluents the concentrations of cortisol and cortisone were measured up to 2.8 ng/L and 0.9 ng/L. Surface water samples contained both steroids in concentrations up to 1.3 ng/L (cortisol) and 1.0 ng/L (cortisone). Moreover, in particular their percentage on the overall GC concentration was found to increase with decreasing wastewater ratios. Both analytes could be detected above LOQ in surface waters upstream of the WWTPs (SW-1a, SW-3a; Tab. A17) without receiving wastewater. This finding indicates these are other sources such as wildlife or agriculture runoff, although these inputs are low compared to the WWTP discharges.

Prescribed quantities of non-halogenated GCs are significantly higher in Germany than those of halogenated steroids (Tab. A14). The measured concentrations of prednisolone and prednisone in treated wastewaters and surface waters did not reflect this consumption quantity. Both analytes were detected in the effluents of WWTP 3 and WWTP 4, whereby concentrations of prednisolone with 0.3 ng/L and 0.6 ng/L found to be slightly higher than prednisone with 0.2 ng/L and 0.4 ng/L. Prednisolone was detected in 17 of 22 rivers and streams above LOQ ranging from 0.05 ng/L to 0.4 ng/L, whereas only two stream (SW-6, SW-9a) contained prednisone above LOQ. Furthermore, budesonide was also found in the effluent of WWTP 3 (1.2 ng/L) and WWTP 4 (2.0 ng/L) and additionally in the corresponding surface water taken downstream from WWTP 3 (0.7 ng/L).

The environmental abundance and application quantities of halogenated and non-halogenated GC, suggest divergent degradation during wastewater treatment. Literature data for the removal efficiencies of most GCs are rare [64, 87, 97]. However, laboratory degradation experiments for a limited number of GCs in contact with activated sludge support this hypothesis [92]. Halogenated GCs were designed to enhance the glucocorticoidal potency. These modifications in their structure lead to stronger binding and higher persistency, which is metabolized less rapidly [110]. Thus, the inhibition of these enzymatic reactions, could also affect the behavior during the municipal wastewater treatment and results in more persistent pollutants with lower degradation rates.

Occurrence of Topically Applied Glucocorticoids. Among other steroids, the ester derivatives of betamethasone and 6α-methylprednisolone are mainly utilized topically in ointments and creams for the medicinal therapy of diverse skin diseases. Although these esters are known to metabolize extensively, researches could show the presence of betamethasone valerate in WWTP effluents [96]. To investigate their presence in water samples, we included the diesters and monoesters of betamethasone and 6α-methylprednisolone in the analytical method. In all WWTP effluents betamethasone propionate, betamethasone valerate and 6α-methylprednisolone propionate exhibited higher concentrations than their alcohols. Measured concentrations of the betamethasone propionate and valerate ester ranged from 0.3 ng/L to 3.6 ng/L and from 1.1 ng/L to 2.5 ng/L, respectively. Non-esterified betamethasone concentrations were between 0.05 ng/L and 0.6 ng/L. The profile of the detected derivatives of 6α-methylprednisolone was similar to betamethasone. Concentrations of 6α-methylprednisolone and its propionate monoester were found up to 1.0 ng/L and 4.2 ng/L in WWTP effluents. Therefore, the results indicated higher abundances of topically applied monoester derivatives than parent alcohol derivatives, thus these monoesters should be considered in further studies. Moreover, esterified steroids are reported to be more potent [106] due to faster diffusion and uptake into the cell, so this might be also affecting the uptake in waterborne organisms. Nevertheless, single betamethasone concentrations were in good agreement to those found in U.S. WWTP effluents (0.18-0.66 ng/L) [83] and Japanese wastewaters (0.29-1.3 ng/L) [94]. However, a substantially higher concentration was measured in one French WWTP effluent (7 ng/L).42 Betamethasone valerate concentrations in the literature (0.84-4.7 ng/L) [94, 96] are comparable to our detected values in WWTP effluents.

Similar to betamethasone valerate, clobetasol propionate is administered topically [54-56]. In WWTP effluents clobetasol propionate concentrations ranged from 0.5 ng/L to 5.4 ng/L. Furthermore, clobetasol propionate was found in 12 of 22 surface water samples above the LOQ, in concentrations ranging up to 3.4 ng/L (SW-3b).

Nonetheless, to our knowledge, this study reveals the first reported concentrations of betamethasone valerate in European water bodies and moreover, the occurrence of betamethasone propionate and 6α-methylprednisolone propionate in notable frequencies and concentrations in treated wastewater as well as rivers and streams is reported for the first time.

Progestogens (PG). Cyproterone acetate and dienogest were found to be the most common detected PG. Highest concentrations were obtained for dienogest, ranging from 1.3 ng/L to 4.4 ng/L in WWTP effluents (Tab. 8) and in 10 of 22 surface water samples from 0.05 ng/L to 2.3 ng/L, respectively. Its metabolite 6β-hydroxy dienogest was present in 4 of the 5 WWTP effluent samples above LOQ (0.6 - 0.9 ng/L). Concentrations of cyproterone acetate ranged from 0.8 ng/L to 3.7 ng/L in WWTP effluents and in 6 of the 22 surface water samples from 0.2 ng/L to 2.6 ng/L. Moreover, further 7 of the investigated 18 PG were found in at least one sample above the detection limits (Tab. A17, e.g. chlormadinone acetate, levonorgestrel, medroxy progesterone acetate). During our sampling campaign levonorgestrel was found in 2 surface waters up to 0.7 ng/L. Available data of PG in environmental waters, especially

of synthetic PG are limited [88], except of levonorgestrel. For example, in 71 French surface water samples, the mean concentration was 3.6 ng/L (detection frequency of 47 %) [89], in Spanish effluents it was frequently found in concentrations up to 4 ng/L [111], whereas only one effluent (1 ng/L)[112] contained levonorgestrel in Germany. These differences might be due to country depending consumption of levonorgestrel.

Impacts of PG on the endocrine systems of aquatic organisms cannot be excluded even if concentrations were found to be in the sub-ng/L range, since toxicological studies described an inhibition of reproduction in fathead minnow exposed to levonorgestrel traces (0.8 ng/L) [69]. A second study showed decreasing testosterone plasma levels in fish when exposed to 1 ng/L cyproterone acetate [113].

In particular, no information with regard to the occurrences and ecotoxicological potentials of dienogest and its hydroxylated metabolite was found in the literature. To our best knowledge, this is the first time that both steroids were found in environmental samples. The ecotoxicological risks to the aquatic environment need to be evaluated in further investigations, since reasonable concentrations were detected in all WWTP effluents (1.3-4.4 ng/L) as well as in rivers and streams (0.05-2.3 ng/L).

To make matters worse, supplemental interactions of steroid mixtures on endocrine systems should be expected as it is known for estrogenic compounds [114]. The assessment of single concentrations may underestimate the total adversary effects from steroidal micropollutants on aquatic organisms. However, the knowledge about single compounds and steroid compositions of concern is still lacking. Mixtures of endocrine active substances are known to acting additive [104], less-than-additive [115] or even show synergistic interactions [116] depending on the organism and composition. Therefore, determining a broad number of steroids by comprehensive and sensitive analytical methods is an important tool for prioritizing compounds of concern and identifying hormone mixtures reaching water bodies. The developed ultra-sensitive multi-method has enabled to successfully identify the predominant steroids and revealed a large number of known, and more importantly, several unknown steroidal pollutants in various surface waters and WWTP effluents.

3.1.4.3 Conclusions

A very sensitive LC-MS-MS for determination of a broad range of hormonal pharmaceuticals in water samples could be established. To realize the required low LOQs a SPE enrichment of a high sample volume and a silica gel clean-up is necessary. Using this method concentrations down to <1 ng/L can be measured even in WWTP effluents. In a small sample campaign WWTP effluent and surface water were analyzed. The results clearly show a widespread occurrence of hormanal pharmaceuticals in WWTP effluent and even in surface water. Highest concentration were found for triamcinolone acetonide with 28 ng/L. Diesters and monoesters of betamethasone and 6α-methylprednisolone show higher concentrations than their alcohols, so the mono und diesters should be considered in environmental monitoring.

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3.1.5 Sediment and suspended particulate matter

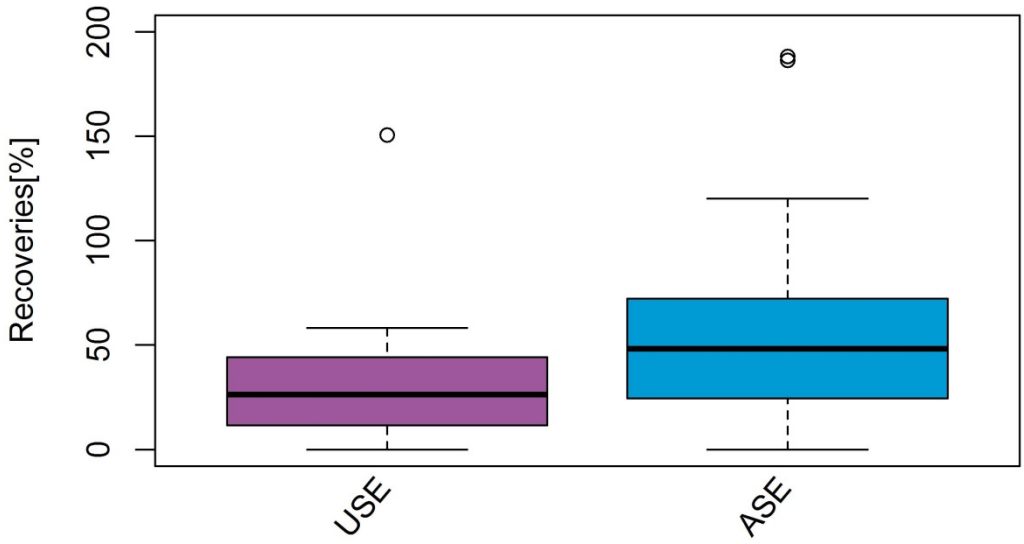
A general literature review about the extraction techniques for pharmaceuticals in sediment and/or suspended particulate matter show that two extraction techniques are mainly used: ultrasonic solid extraction (USE) and accelerated solvent extraction (ASE). In consequence, we tested both methods while applying conditions determined from the literature review.

For the ultrasonic solid extraction, 1 g sediment was extracted. The extraction solvent was methanol/water 50/50, v/v and 3 extraction cycles were performed with 10 mL solvent and duration of 15 minutes respectively. The supernatant were put together and evaporated to dryness before dissolution and injection in LC-MS/MS.

For the accelerated solvent extraction, 1 g sediment was also extracted. Methanol/water, 50/50 (v/v) was also used as an extraction solvent. The extraction temperature was set up to 80 °C to limit possible degradation of the analytes and 3 extraction cycles of 15 minutes were performed.

The extraction recoveries obtained with each method were then compared (Fig. 15), ASE yielded significantly better extraction recoveries and was thus select for further development.

Figure 15: Boxplots of the recoveries of the extraction procedure with ultrasonic extraction and accelerated solvent extraction. . (Source: Own representation, Federal Institute of Hydrology)

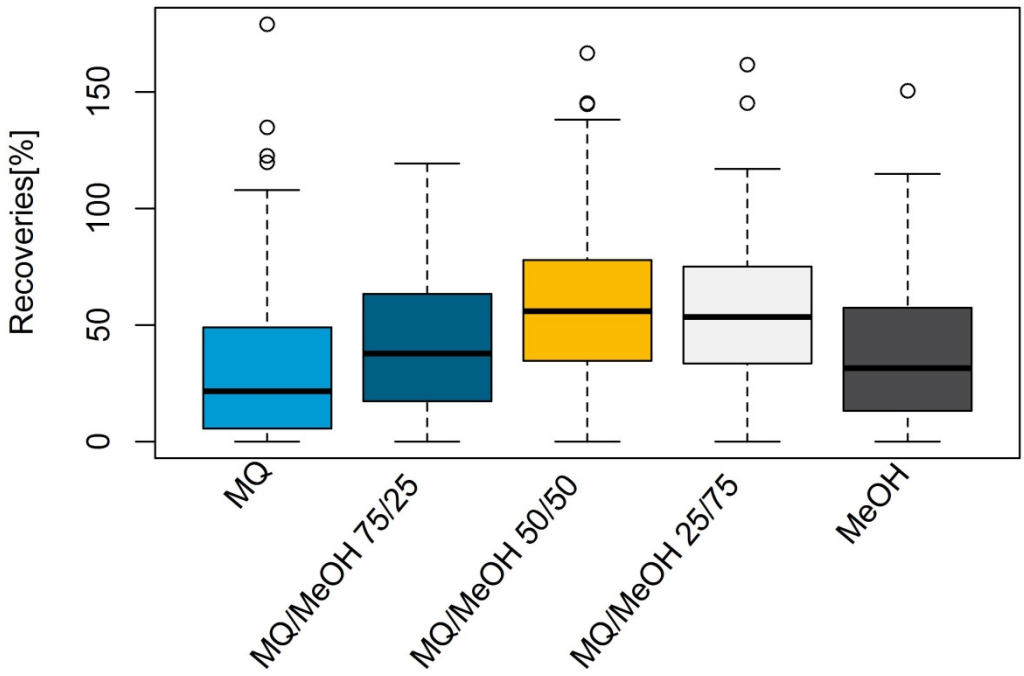


Stability of the analytes. In a second step, the stability of the molecules during the ASE procedure was tested by the realization of the extraction procedure in sea sand. Most of the molecules were stable for extraction at temperature from 80 to 120 °C. However all N-Oxide and S-Oxide metabolites/transformation products were totally degraded as well as ranitidine and azithromycin. Ibuprofen, atenolol acid and 7-hydroxyquetiapine were also partly degraded even at 80°C. From 100 °C, others molecules begin to be partly degraded too, as for example clopidogrel or diphenhydramine.

Optimization of the extraction conditions for the ASE. All following extraction conditions were optimized with sediment from Ehrenbreitstein, spiked at 7.5 ng/g. The amount of sediment extracted was 1 g. As recoveries, it is understood recoveries over the extraction procedure with correction of the matrix effect (e.g. internal standard were added before the LC-analysis).

The composition of the extraction solvent is one of the major factors in optimization of efficient ASE procedure. Literature review shows that most of the authors use a mixture of water and a relative polar organic solvent (mostly methanol) to perform the extraction of pharmaceuticals from sediment [48-53]. Thus, we first compared the recoveries obtained with water, water/methanol (75:25), water/methanol (50:50), water/methanol (25:75) and methanol. The extraction solvent water/methanol (1:1) provides the best recoveries overall (Fig. 16).

Figure 16: Boxplot of the recoveries obtained with different extraction solvents. MQ: Milli-Q, MeOH: methanol. (Source: Own representation, Federal Institute of Hydrology)



The pH of the extraction solvent influences the charge state of the analytes and thus their sorption ability. Pre-test shows that a basic extraction solvent does not yield better recoveries than a neutral solvent (data not shown). On the contrary, the addition of acid to the extraction solvent improves the extraction significantly for 14 analytes. Further experiments were made to evaluate the optimal pH, it was observed that pH between 2 and 3 provided the optimal extraction with no much influence of the pH in this range (Fig. 17). To avoid too high concentration of acid, which can alter the MS signal later on, a concentration of 0.1 % in formic acid (pH 2.5) was chosen.

Figure 17: Boxplot of the recoveries obtained for different pH for the aqueous part of the extraction solvent methanol/water, 50/50, v/v. (Source: Own representation, Federal Institute of Hydrology)

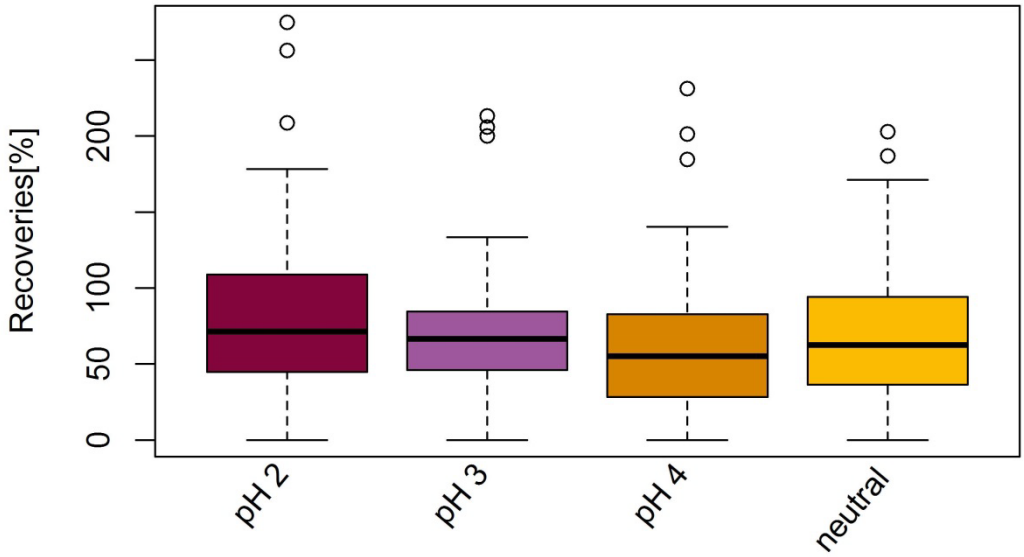
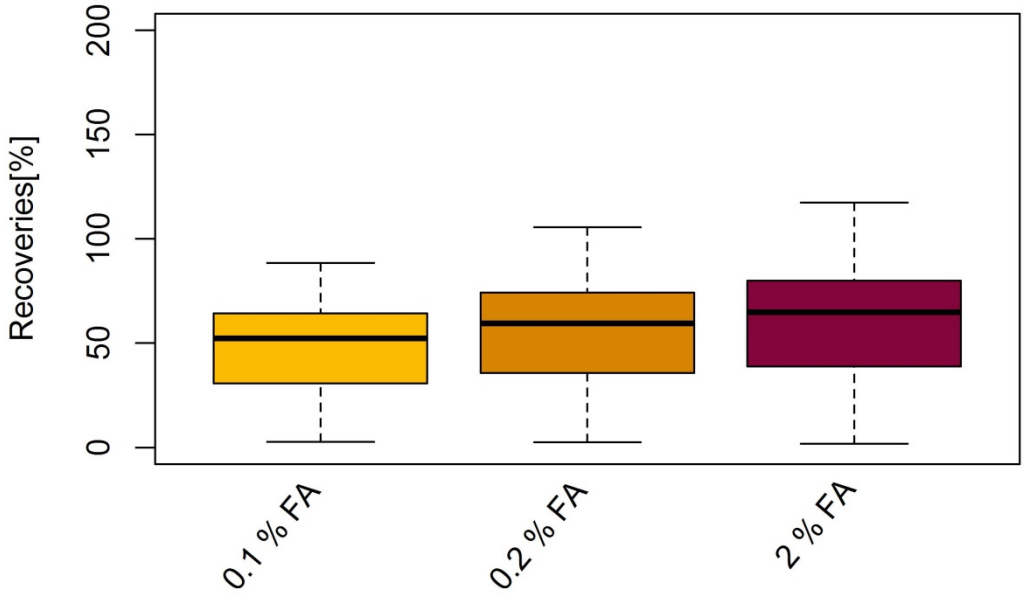
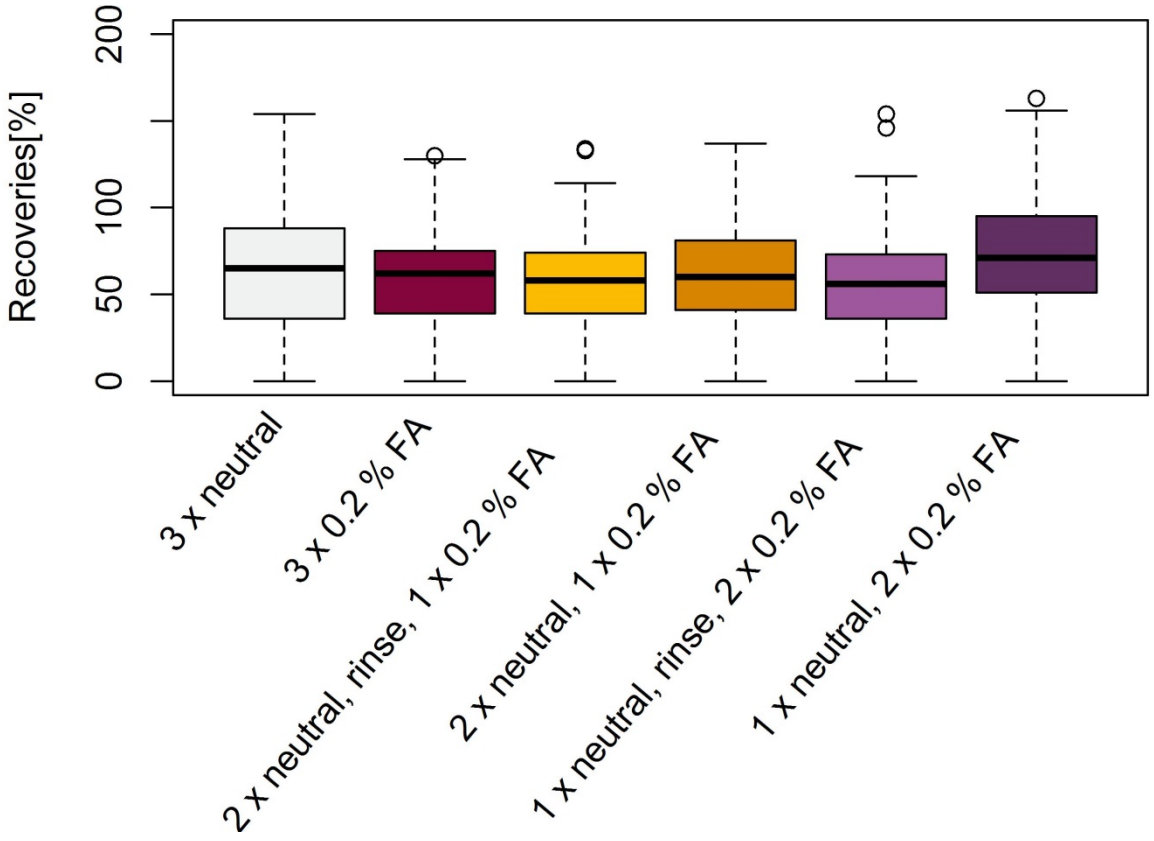


Figure 18: Boxplot of the recoveries for different volumetric percentage of formic acid in the aqueous phase of the extraction solvent, MeOH/MQ, 50/50, v/v.. Approximate corresponding pH : 2.7, 2.5,2.0. (Source: Own representation, Federal Institute of Hydrology)



As some compounds were better extracted by neutral solvent it was decided to use two different solvents for the extraction: MeOH/Milli-Q (50/50) and MeOH/0.2 % formic acid (50/50). It was then shown that the best overall recoveries could be obtained with a first extraction cycle with the neutral solvent and two subsequent cycles with acidified eluent without rinse step in between. The absence of rinse step causes probably a pH-gradient bringing about the best efficiency of extraction.

Figure 19: Boxplots of the recoveries obtained with different procedure, the number correspond to the static cycles, neutral to the extraction solvent MeOH/MQ, 50/50 and 0.1 % FA to the extraction solvent MeOH/0.2 % formic acid, 50/50. (Source: Own representation, Federal Institute of Hydrology)



Conclusion. ASE shows the best extraction efficiency for the tested pharmaceuticals from particular matter and sediment samples. For extraction of basic and acid compounds two extraction steps using a neutral and an acidic solvent are necessary. Recoveries of the whole range of tested pharmaceuticals are sufficient for environmental analysis. But validation of the method has to be finished before the first sample could be analyzed.

3.2 Conception of a monitoring program

In co-work with the UBA sampling locations for a monitoring program in limnic systems were selected. The selected limnic systems are shown in Table 11. They represent surface waters with different effluent affection. From all six sampling sites water, suspended particular matter and biota samples should be taken. In case of water samples mixed samples over a periode of one weak or grab samples from five consecutive days are recommended. Sample pretreatments for pharmaceuticals and hormonal pharmaceuticals are described in section 3.1.2.1 and 3.1.4.1, respectively.

Suspended particular matter and biota samples are provided by the German environmental specimen bank. Except for the sampling site Nidda, where sampling was performed by the BfG. Due to their fat content (~9%), feeding habits and the availability of comparison specimen for time trend analysis breams (liver and filets) were chosen for biota analysis. Sampling procedure and sample pretreatment are described in standard operation guidelines of the German environmental specimen bank [119, 120].

Table 11: Sample sites

No.	sampling site	effluent affection	Water	Particular matter	Biota (bream)
1	Rhine	low	yes	yes	yes
2	Nidda	low	yes	yes	no
3	Saar	medium	yes	yes	yes
4	Berlin Unterhavel	high	yes	no	no
5	Mühlbach	high	yes	no	no
6	Lake Stechlin (reference)	no	yes	no	no

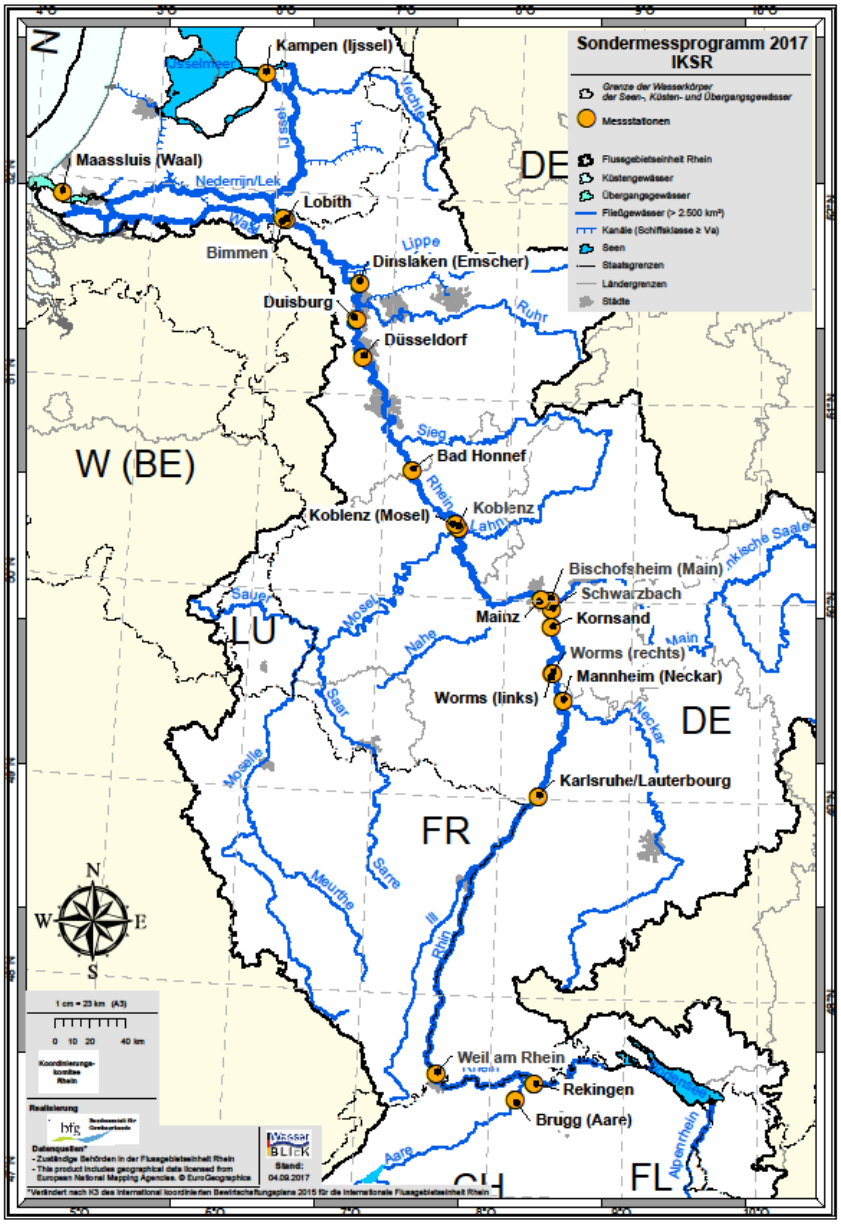
4 Work package 3: Environmental monitoring

In a small sampling campaign water samples from different surface waters were taken in 2016 and 2017 and analyzed using the new quantification methods.

4.1 Rhine

Water samples from the river Rhine were provided from the monitoring program of the ICPR (international commission for the protection of the river Rhine). These are one week composite samples taken from all along the Rhine (Fig. 20).

Figure 20: Map of the sampling locations along the Rhine. (Source: Tom Gemüth, Federal Institute of Hydrology)



Quantification results reveal an increasing pollution along the Rhine. Two main discharge places could be identified: Schwarzbach and Dinslaken. No particular industry discharge could be identified. All analytes except paracetamol and mesalazine N-acetyl were detected at least in one sample. In the Schwarzbach and Dinslaken samples, respectively 23 and 24 analytes over 27 were detected. The cleanest samples were those taken upstream in Switzerland (Brugg-Aare and Rekingen am Rhein) but even in these samples 12 of the 27 analytes were detected with concentrations up to 910 ng/L for guanyl urea. In the most loaded samples (Dinslaken), 8 analytes show concentrations over 1 µg/L with 19.3 µg/L as highest concentration for guanyl urea. The hormonal pharmaceuticals show the same tendency (ref. Table 13). Highest concentrations could be found at sample site Dinslaken. Incept of halcinoide all of the analytes could be found and concentrations of triamcinolone aceteonide and canrenone were as high as 11 ng/L.

Table 12: Concentrations of the analytes detected in the different Rhine sample in ng/L

	Concentration [ng/L]																			
	Brugg-Aare	Rekingen am Rhein	Weil am Rhein	Karlsruhe/Lauterbourg	Mannheim (Neckar)	Worms (Linksrh.)	Worms (Rechtrh.)	Kornsand (Rechtsrh.)	Schwarzbach (Trebur, Hessen)	Bischofsheim	Mainz (Leitung 2)	Koblenz-Rhein	Koblenz-Mosel	Bad Honnef	Dusseldorf-Flehe (rechtsrh.)	Duisburg (linksrh.)	Dinslaken (Emschermündung)	Lobith	Bimmen	Maassluis	Kampen
4-Acetamidoantipyrine	95 ± 1	52.8 ± 0.8	80.5 ± 0.7	80.9 ± 0.5	90 ± 1	106 ± 1	150.9 ± 0.8	119 ± 2	1090 ± 20	296 ± 3	111 ± 2	140 ± 2	87 ± 2	151 ± 2	167.8 ± 0.6	150.1 ± 0.7	2960 ± 10	194 ± 4	161 ± 1	136 ± 3	176 ± 7
4-Formylaminoantipyrine	49 ± 1	32 ± 2	47 ± 1	58 ± 2	73 ± 7	90 ± 20	160 ± 30	100 ± 2	1500 ± 100	390 ± 70	97 ± 3	132 ± 4	81 ± 2	170 ± 30	182 ± 2	220 ± 50	2300 ± 200	260 ± 60	174 ± 4	146 ± 5	200 ± 10
4-Methylaminoantipyrine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	2.5 ± 0.4	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	9 ± 1	<LOQ	<LOQ	<LOQ	<LOQ
9-Acridine carboxylic acid	2.62 ± 0.09	3.9 ± 0.9	2.47 ± 0.08	2.15 ± 0.06	2.4 ± 0.1	2.32 ± 0.06	4.1 ± 0.2	3.49 ± 0.05	35 ± 2	8.8 ± 0.3	2.61 ± 0.07	4.0 ± 0.2	2.76 ± 0.05	3.71 ± 0.03	3.7 ± 0.1	3.9 ± 0.1	21 ± 3	4.4 ± 0.2	4.2 ± 0.3	3.5 ± 0.1	5.3 ± 0.2
Abacavir	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.97 ± 0.01	<LOQ	<LOQ	<LOQ	<LOQ
Abacavir carboxylate	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	5 ± 1	<LOQ	25 ± 2	10 ± 3	<LOQ	<LOQ	<LOQ	4.8 ± 0.8	5.3 ± 0.3	5.4 ± 0.9	8 ± 2	5.5 ± 0.8	<LOQ	<LOQ	<LOQ
Acesulfame	480 ± 20	342 ± 10	432 ± 9	447 ± 6	460 ± 10	500 ± 20	615 ± 9	500 ± 10	4200 ± 200	1060 ± 20	510 ± 20	580 ± 10	550 ± 30	610 ± 20	620 ± 30	610 ± 9	12100 ± 800	850 ± 30	700 ± 20	740 ± 50	890 ± 30
Acyclovir	4.06 ± 0.08	2.27 ± 0.09	4.2 ± 0.1	4.28 ± 0.03	5.37 ± 0.07	9.1 ± 0.2	8.43 ± 0.08	7.6 ± 0.2	27.6 ± 0.6	22.7 ± 0.5	2.98 ± 0.08	7.3 ± 0.1	8.32 ± 0.08	22.6 ± 0.5	8.6 ± 0.1	10.9 ± 0.2	70 ± 2	8.95 ± 0.08	8.9 ± 0.2	14.4 ± 0.4	7.4 ± 0.4
Bisoprolol	1.4 ± 0.01	1.13 ± 0.01	1.89 ± 0.01	3.1 ± 0.03	3.86 ± 0.06	4.79 ± 0.06	7.24 ± 0.03	6 ± 0.2	419 ± 5	31.2 ± 0.2	5.34 ± 0.08	8.36 ± 0.05	8.57 ± 0.04	9.82 ± 0.1	12.7 ± 0.3	11.52 ± 0.07	271 ± 4	15.9 ± 0.2	11.7 ± 0.2	12 ± 0.1	14.3 ± 1

	Concentration [ng/L]																			
	Brugg-Aare	Rekingen am Rhein	Weil am Rhein	Karlsruhe/Lauterbourg	Mannheim (Neckar)	Worms (Linksrh.)	Worms (Rechtrh.)	Kornsand (Rechtsrh.)	Schwarzbach (Trebur, Hessen)	Bischofsheim	Mainz (Leitung 2)	Koblenz-Rhein	Koblenz-Mosel	Bad Honnef	Dusseldorf-Flehe (rechtsrh.)	Duisburg (linksrh.)	Dinslaken (Emschermündung)	Lobith	Bimmen	Maassluis	Kampen
Clindamycin	1.29 ± 0.02	1.17 ± 0.03	1.49 ± 0.01	1.91 ± 0.02	2.39 ± 0.05	3.11 ± 0.06	4.79 ± 0.09	3.02 ± 0.03	92 ± 5	10.2 ± 0.9	3.26 ± 0.06	5 ± 0.1	13.9 ± 0.3	5.83 ± 0.01	6.6 ± 0.2	5.9 ± 0.8	90 ± 2	8.4 ± 0.3	8.7 ± 0.7	5.85 ± 0.04	8 ± 0.4
Clindamycin sulfoxide	2.44 ± 0.07	3 ± 0.2	3 ± 0.1	3.3 ± 0.1	4.4 ± 0.1	6.0 ± 0.4	11.1 ± 0.4	5.7 ± 0.3	133 ± 7	24 ± 3	5 ± 1	8.4 ± 1	8.7 ± 0.4	10 ± 2	13.2 ± 0.5	14 ± 1	200 ± 20	15 ± 2	11.5 ± 0.5	13 ± 1	14.4 ± 0.6
Diatrizoate	21 ± 1	33 ± 2	34 ± 2	39 ± 1	48 ± 5	69 ± 4	145 ± 2	120 ± 5	4400 ± 400	420 ± 20	113 ± 7	154 ± 7	140 ± 10	167 ± 6	210 ± 9	220 ± 20	2900 ± 600	250 ± 30	212 ± 2	180 ± 10	290 ± 70
Emtricitabine	<LOQ	<LOQ	0.6 ± 0.1	<LOQ	<LOQ	0.7 ± 0.1	<LOQ	<LOQ	18.2 ± 0.2	0.6 ± 0.06	0.64 ± 0.04	0.49 ± 0.01	<LOQ	0.44 ± 0.07	<LOQ	0.64 ± 0.07	35 ± 3	0.69 ± 0.05	<LOQ	1.15 ± 0.09	<LOQ
Emtricitabine carboxylate	13.3 ± 0.2	<LOQ	12.8 ± 0.8	12 ± 1	16 ± 1	24 ± 2	27 ± 3	17 ± 2	190 ± 40	40 ± 10	13 ± 5	21 ± 2	<LOQ	18 ± 6	47 ± 6	35 ± 7	6.9 ± 0.6	30 ± 4	22 ± 2	<LOQ	38 ± 8
Emtricitabine S-oxide	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	48 ± 6	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Gabapentin	116 ± 5	131 ± 7	125 ± 5	140 ± 4	180 ± 10	210 ± 10	320 ± 20	210 ± 20	1900 ± 500	770 ± 40	190 ± 20	290 ± 10	300 ± 10	320 ± 40	430 ± 50	450 ± 30	1900 ± 400	590 ± 30	400 ± 20	93 ± 6	620 ± 30
Gabapentin lactam	8.4 ± 0.1	12 ± 0.3	10.3 ± 0.3	14.6 ± 0.4	17.7 ± 0.2	22.6 ± 0.7	37 ± 2	27.1 ± 0.8	840 ± 10	98 ± 1	25.7 ± 0.1	34.2 ± 0.5	34 ± 1	44.78 ± 0.04	48.1 ± 0.4	58.3 ± 0.6	630 ± 20	62.43 ± 0.07	55 ± 0.5	45 ± 1	86 ± 5
Lamivudine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	3.4 ± 0.1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	5.1 ± 0.1	<LOQ	<LOQ	<LOQ	<LOQ
Metformin	320 ± 30	200 ± 30	272 ± 9	270 ± 20	320 ± 60	430 ± 80	480 ± 70	370 ± 20	1100 ± 200	900 ± 200	370 ± 20	420 ± 20	790 ± 40	500 ± 100	520 ± 10	500 ± 80	4400 ± 700	660 ± 90	450 ± 30	350 ± 20	466 ± 6
Guanyl urea	910 ± 30	340 ± 20	690 ± 30	460 ± 10	580 ± 60	940 ± 50	1400 ± 100	810 ± 70	11000 ± 2000	3100 ± 30	16 ± 4	960 ± 40	1110 ± 80	980 ± 50	1430 ± 80	1070 ± 60	19300 ± 500	1640 ± 40	940 ± 10	520 ± 50	1220 ± 50
N-acetyl mesala-	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LO	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

	Concentration [ng/L]																			
	Brugg-Aare	Rekingen am Rhein	Weil am Rhein	Karlsruhe/Lauterbourg	Mannheim (Neckar)	Worms (Linksrh.)	Worms (Rechtsrh.)	Kornsand (Rechtsrh.)	Schwarzbach (Trebur, Hessen)	Bischofsheim	Mainz (Leitung 2)	Koblenz-Rhein	Koblenz-Mosel	Bad Honnef	Dusseldorf-Flehe (rechtsrh.)	Duisburg (linksrh.)	Dinslaken (Emschermündung)	Lobith	Bimmen	Maassluis	Kampen
zine										Q											
Oxipurinol	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	310 ± 30	<LOQ	4800 ± 400	880 ± 70	<LOQ	190 ± 40	260 ± 20	<LOQ	<LOQ	320 ± 30	6000 ± 2000	190 ± 20	<LOQ	700 ± 200	500 ± 10
Paracetamol	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Ranitidine	1.1 ± 0.1	0.46 ± 0.01	1.08 ± 0.02	0.98 ± 0.03	1.1 ± 0.03	1.95 ± 0.02	2.6 ± 0.05	1.21 ± 0.01	32.8 ± 0.6	9.9 ± 0.2	1.7 ± 0.05	2.32 ± 0.06	2.19 ± 0.03	2.57 ± 0.02	4.15 ± 0.03	3.31 ± 0.04	177 ± 2	5.85 ± 0.09	3.93 ± 0.05	4.29 ± 0.08	5.5 ± 0.3
Desmethyl raniti-dine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.5 ± 0.5	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	4.8 ± 0.8	<LOQ	<LOQ	<LOQ	<LOQ
Ranitidine N-oxide	<LOQ	3.47 ± 0.02	3.48 ± 0.01	<LOQ	3.47 ± 0.01	3.48 ± 0.03	3.59 ± 0.05	3.51 ± 0.01	4.16 ± 0.08	3.9 ± 0.2	3.5 ± 0.01	<LOQ	<LOQ	3.54 ± 0.07	3.57 ± 0.01	3.54 ± 0.03	3.8 ± 0.1	3.57 ± 0.06	3.5 ± 0.01	<LOQ	3.5 ± 0.01
Ranitidine S-oxide	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	2.44 ± 0.04	2.68 ± 0.01	2.46 ± 0.08	14.3 ± 0.9	4.8 ± 0.3	2.47 ± 0.06	2.62 ± 0.03	2.39 ± 0.04	2.6 ± 0.1	2.9 ± 0.1	2.9 ± 0.2	6 ± 1	3.1 ± 0.1	2.81 ± 0.05	2.45 ± 0.06	2.86 ± 0.02

Table 13: Concentrations of the hormonal pharmaceuticals detected in the different Rhine sample in ng/L

Substance	Brugg-Aare (P1)	Rekingen P1	Weil a. R. P1	Weil a. R. P2	Karlsruhe P1	Neckar P1	Worms P1 (links)	Worms P1 (rechts)	Kornsand P1	Kornsand P2(whitin 24h)	Schwarzbach P1	Bischofsheim P1
Mineralocorticoids (MC)												
Canrenone	0.1	<LOQ	0.1	0.1	0.1	0.20	0.2	0.3	0.2	0.2	2.9	0.6
7a-Thiomethyl sironolactone	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.03	0.04	<LOQ	<LOQ	0.3	0.07
Glucocorticoids (GC)												
6b-Hydroxy triamcinolone acetonide	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.05	<LOQ	<LOQ	0.5	0.1
Beclomethasone	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.05	<LOD
Betamethasone	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.08	<LOD	<LOD	1.2	0.2
ΣBetamethasone propionate	<LOD	<LOD	<LOQ	<LOQ	<LOD	<LOD	<LOQ	<LOQ	<LOD	<LOQ	0.2	0.06
ΣBetamethasonvalerat	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD
Budesonide	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Clobetasol propionate	0.04	<LOQ	<LOQ	0.06	<LOQ	<LOQ	0.05	0.06	<LOQ	<LOD	0.3	0.1
Cortisol	0.04	0.04	0.09	0.2	0.06	0.07	0.1	0.07	0.07	0.5	0.4	0.2
Cortisone	<LOQ	0.05	0.1	0.08	0.04	0.06	0.2	<LOQ	0.05	0.4	0.1	0.1
Fluocinolone acetonide	<LOD	<LOQ	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.07	<LOQ
Fluticasone propionate	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.1	<LOQ
Halcinonide	0.03	<LOD	<LOQ	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Methylprednisolone	<LOQ	<LOD	<LOQ	<LOD	<LOD	0.00	<LOQ	<LOQ	<LOD	<LOD	0.1	0.05
ΣMethylprednisolone propionate	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.20	<LOD
Mometasone furoate	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Prednisolone	0.50	0.30	<LOQ	0.04	0.08	0.24	<LOQ	<LOQ	0.08	0.08	0.2	0.1
Prednisone	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.04	<LOD
Triamcinolone acteonide	<LOD	<LOQ	<LOQ	<LOQ	0.05	0.07	0.1	0.2	0.09	0.06	4.0	0.7
Progestogens (PG) & Estrogens												
6b-Hydroxy dienogest	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD
Cyproterone acetate	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	0.6	<LOD
Dienogest	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	0.4	0.2
Estrone	0.07	<LOD	0.08	0.3	0.09	0.06	0.06	0.05	0.08	0.5	0.7	0.2
Levonorgestrel	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

Substance	Mainz (Leitung 2) P1	Mosel P1	Bad Honnef P1	Duisburg P1	Dinslaken P1	Lobbith P1	Bimmen P1	Kampen P1	Maassluis P1
Mineralocorticoids (MC)									
Canrenone	0.3	0.3	0.2	0.4	11	0.4	0.3	0.4	0.4
7a-Thiomethyl sironolactone	<LOQ	0.03	0.03	0.04	1.2	0.06	0.03	0.04	0.03
Glucocorticoids (GC)									
6b-Hydroxy triamcinolone acetonide	<LOQ	<LOQ	<LOQ	0.06	2.0	0.06	<LOQ	0.06	<LOQ
Beclomethasone	<LOD	<LOD	<LOD	<LOD	0.09	<LOD	<LOD	<LOD	<LOD
Betamethasone	<LOD	0.06	0.07	0.05	1.3	0.1	0.08	0.06	0.1
ΣBetamethasone propionate	<LOD	0.05	<LOQ	<LOQ	0.6	<LOD	<LOD	<LOQ	<LOQ
ΣBetamethasonvalerat	<LOD	<LOD	<LOD	<LOD	0.3	<LOD	<LOD	<LOD	<LOD
Budesonide	<LOD	<LOD	<LOD	<LOD	1.1	<LOD	<LOD	<LOD	<LOD
Clobetasol propionate	<LOD	0.06	0.06	0.05	1.8	0.06	0.07	0.06	0.06
Cortisol	0.1	0.2	0.1	0.09	0.4	0.2	0.08	0.09	0.1
Cortisone	0.1	0.1	0.06	0.05	0.1	0.07	<LOQ	<LOQ	0.07
Fluocinolone acetonide	<LOD	<LOD	<LOQ	<LOD	0.07	<LOQ	<LOD	<LOD	<LOD
Fluticasone propionate	<LOQ	<LOD	<LOD	<LOD	0.3	<LOD	<LOD	<LOQ	<LOQ
Halcinonide	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Methylprednisolone	<LOQ	<LOD	<LOQ	<LOD	0.5	<LOQ	<LOD	<LOQ	<LOQ
ΣMethylprednisolone propionate	<LOD	<LOD	<LOD	<LOD	1.0	<LOD	<LOD	<LOD	<LOD
Mometasone furoate	<LOD	<LOD	<LOD	<LOD	0.5	<LOD	<LOD	<LOD	<LOD
Prednisolone	<LOQ	0.08	0.6	0.5	0.1	<LOQ	<LOQ	<LOQ	<LOQ
Prednisone	<LOD	<LOD	<LOD	<LOD	0.5	<LOD	<LOD	<LOD	<LOD
Triamcinolone acteonide	0.1	0.1	0.2	0.2	11	0.4	0.3	0.4	0.3
Progestogens (PG) & Estrogens									
6b-Hydroxy dienogest	<LOD	<LOD	<LOD	<LOD	0.3	<LOD	<LOD	<LOD	<LOD
Cyproterone acetate	<LOD	<LOQ	<LOD	<LOD	2.4	<LOD	<LOD	0.1	<LOQ
Dienogest	<LOD	<LOD	<LOQ	<LOQ	2.0	0.07	<LOQ	<LOD	<LOD
Estrone	0.2	0.2	0.1	0.2	0.7	0.2	0.1	0.2	0.3
Levonorgestrel	<LOD	<LOD	<LOD	<LOD	0.3	<LOD	<LOD	<LOD	<LOD

4.2 Occurrence of pharmaceuticals in surface waters

To evaluate the occurrence of pharmaceuticals in surface water affected by different amounts of wastewater water samples from Lake Stechlin, Nidda, Saar, Rhine, Mühlbach (Groß-Gerau, Hessen) and Teltow Canal were analyzed. The Lake Stechlin is not affected by wastewater and only marginal affected by other pollution routes. In contrast, the Teltow Canal is highly affected by effluents of waste water treatment plants and industrial discharge. The other waters have waste water affections between these extrema.

Samples from Lake Stechlin and Nidda were the less contaminated samples with only 7 and 11 compounds detected with the HILIC-MS/MS method (26 %) and 8 and 12 with the RPLC-MS/MS method (7 %), respectively. The Nidda samples were taken upstream before the WWTP discharge what explained their low contamination level. In the Lake Stechlin and in the Nidda samples, the highest concentrations were measured for metformin with 100 ng/L and 230 ng/L, respectively. In the Teltow Canal samples, the highest concentrations were measured for oxipurinol with 12 µg/L and for the middle polar method for valsartanic acid with 4.6 µg/L. The Mühlbach was sampled before WWTP discharge, at the level of WWTP discharge and a few hundred meters afterwards. Interestingly, the stream was already quite contaminated before the WWTP discharge. Still, 17 additional analytes could be detected after the WWTP discharge. Moreover, for the micropollutants which were already detected before the WWTP discharge the concentrations increase considerably. For example, the concentration in diatrizoate increases from 0.95 µg/L to 17 µg/L and concentration of valsartanic acid from 800 to 5700 ng/L.

Concerning the hormonal pharmaceuticals (Table 17) in the Lake Stechlin only five of the substances could be found. Highest concentrations were found for cortisol (0.2 ng/L) and estrone (0.2 ng/L). On the contrary all substances except prednisolone could be detected in the Teltow Canal. Highest concentrations were found for triamcinolone acetonide (7.6 ng/L) and canrenone (2.9 ng/L). Similar or even higher concentrations were measured in the Mühlbach downstream the WWTP. Highest concentration was found for canrenone with 11 ng/L.

Table 14: Proportion of the analyte detected in the different matrices with RPLC and the HILIC method. In parenthesis, the absolute number of analytes detected.

	Lake Stechlin	Teltow Canal	Saar (Rehlingen)	Nidda N1	Nidda N2	Mühlbach before WWTP	Mühlbach WWTP	Mühlbach after WWTP	Rhine Koblenz
HILIC method	22 (6)	78 (21)	52 (14)	11 (3)	11 (3)	56 (15)	70 (19)	70 (19)	48 (13)
RPLC method	8 (8)	65 (68)	52 (54)	12 (12)	12 (12)	52 (54)	65 (68)	64 (67)	25 (26)

Table 15: Concentration of the extreme polar analytes detected with the HILIC-MS/MS method in the surface water samples

Analytes	Concentration [ng/L]								
	Lake Stechlin	Teltow Canal	Saar (Rehlingen)	Nidda N1	Nidda N2	Mühlbach before WWTP	Mühlbach WWTP	Mühlbach after WWTP	Rhine (Koblenz)
4-Acetamidoantipyrine	<LOQ	860 ± 10	145 ± 1	14.4 ± 0,5	13.1 ± 0,5	357 ± 3	4200 ± 200	4230 ± 80	159 ± 2
4-Formylaminoantipyrine	3 ± 1	3250 ± 30	119 ± 3	<LOQ	<LOQ	400 ± 30	3500 ± 200	3500 ± 100	132 ± 2
4-Methylaminoantipyrine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	strea	308 ± 5	<LOQ
9-Acridine carboxylic acid	<LOQ	155 ± 4	7.2 ± 0,4	<LOQ	<LOQ	26 ± 2	91 ± 3	94 ± 5	5.3 ± 0.4
Abacavir	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Abacavir carboxylate	<LOQ	1030 ± 10	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Acesulfame	44 ± 9	620 ± 30	470 ± 10	49 ± 3	51.7 ± 0,7	1110 ± 40	7900 ± 200	8100 ± 100	322 ± 4
Acyclovir	<LOQ	480 ± 10	18.8 ± 0.7	<LOQ	<LOQ	<LOQ	97 ± 4	92 ± 4	12.4 ± 0.8
Bisoprolol	<LOQ	106 ± 2	16.97 ± 0,07	<LOQ	<LOQ	23.7 ± 0,1	126 ± 3	131 ± 1	8.1 ± 0.3
Clindamycin	<LOQ	89 ± 1	<LOQ	<LOQ	<LOQ	21.8 ± 0,5	150 ± 6	154 ± 1	<LOQ
Clindamycin sulfoxide	<LOQ	207 ± 2	23.2 ± 0,9	<LOQ	<LOQ	56 ± 0.7	500 ± 60	488 ± 7	8.4 ± 0.4
Diatrizoate	13.8 ± 0,7	1760 ± 50	190 ± 3	<LOQ	<LOQ	950 ± 20	16600 ± 800	17500 ± 300	120 ± 2
Emtricitabine	<LOQ	71 ± 2	<LOQ	<LOQ	<LOQ	<LOQ	36 ± 1	34 ± 3	<LOQ
Emtricitabine carboxy-late	<LOQ	1090 ± 20	<LOQ	<LOQ	<LOQ	<LOQ	400 ± 20	383 ± 10	<LOQ
Emtricitabine *S*-oxide	<LOQ	310 ± 50	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Gabapentin	<LOQ	745 ± 6	243 ± 9	<LOQ	<LOQ	350 ± 10	2400 ± 200	2400 ± 60	158 ± 7
Gabapentin lactam	27.9 ± 0.4	200 ± 5	<LOQ	<LOQ	<LOQ	211 ± 3	1110 ± 30	1120 ± 30	<LOQ
Lamivudine	<LOQ	27.3 ± 0,9	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Metformin	100 ± 10	980 ± 90	1080 ± 50	230 ± 30	195 ± 2	2100 ± 100	1650 ± 70	1500 ± 100	940 ± 10

Analytes	Concentration [ng/L]								
	Lake Stechlin	Teltow Canal	Saar (Rehlingen)	Nidda N1	Nidda N2	Mühlbach before WWTP	Mühlbach WWTP	Mühlbach after WWTP	Rhine (Koblenz)
Guanyl urea	64 ± 3	5910 ± 20	992 ± 2	<LOQ	<LOQ	1314 ± 8	18600 ± 600	19000 ± 200	574 ± 3
N-acetyl mesalazine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Oxipurinol	<LOQ	12200 ± 700	440 ± 60	<LOQ	<LOQ	1810 ± 20	16000 ± 2000	16000 ± 1000	290 ± 40
Paracetamol	<LOQ	<LOQ	92 ± 3	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Ranitidine	<LOQ	32,2 ± 0,3	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Desmethyl ranitidine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Ranitidine *N*-oxide	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	21.95 ± 0,09	22.3 ± 0,1	22.37 ± 0,06	<LOQ
Ranitidine *S*-oxide	<LOQ	14 ± 1	4.8 ± 0,06	<LOQ	<LOQ	12.39 ± 0,08	92 ± 6	88.4 ± 0,7	1.66 ± 0.03

Table 16: Concentrations of the medium polar analytes detected with the RPLC-Method

Analyte	Concentration [ng/L]								
	Lake Stechlin	Teltow Canal	Saar (Rehlingen)	Nidda N1	Nidda N2	Mullbach before WWTP	Mullbach WWTP	Mullbach after WWTP	Rhine (Koblenz)
Aliskiren	<LOQ	75 ± 1	17 ± 0.6	<LOQ	<LOQ	43 ± 6	530 ± 20	510 ± 10	<LOQ
Amisulpride	<LOQ	312 ± 4	14.6 ± 0.7	2.3 ± 0.2	1.4 ± 0.1	34.2 ± 0.9	500 ± 20	510 ± 6	10.1 ± 0.4
O-Desmethyl amisulpride	0.4 ± 0.6	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Aripiprazole	<LOQ	<LOQ	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	<LOQ
Aripiprazole N1-Oxide	<LOQ	<LOQ	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	<LOQ
Dehydroaripiprazole	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Atenolol	<LOQ	36 ± 0.7	<LOQ	<LOQ	<LOQ	<LOQ	117 ± 5	120 ± 10	<LOQ
Atenolol acid	<LOQ	169 ± 3	111 ± 5	<LOQ	<LOQ	208 ± 7	1130 ± 70	1210 ± 60	65 ± 3
Hydroxyatenolol	<LOQ	3.4 ± 0.6	<LOQ	<LOQ	<LOQ	<LOQ	8.1 ± 0.7	8 ± 1	<LOQ
Bezafibrate	<LOQ	38.9 ± 0.3	13 ± 1	<LOQ	<LOQ	13.2 ± 1	139.9 ± 0.2	170 ± 20	8.5 ± 0.5

Analyte	Concentration [ng/L]								
	Lake Stechlin	Teltow Canal	Saar (Rehlingen)	Nidda N1	Nidda N2	Mullbach before WWTP	Mullbach WWTP	Mullbach after WWTP	Rhine (Koblenz)
3-[(4-chlorobenzoyl) amino]propanoic acid	n.a.	n.a.	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Bilcalutamide	<LOQ	26.7 ± 0.6	2.9 ± 0.08	<LOQ	<LOQ	4.6 ± 0.2	39 ± 2	39.9 ± 0.6	n.a.
Hydroxylbosentan	<LOQ	<LOQ	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	<LOQ
Candesartan	<LOQ	610 ± 3	67 ± 1	2.2 ± 0.2	2.02 ± 0.01	251 ± 6	2030 ± 60	2080 ± 20	43 ± 3
Carbamazepine	<LOQ	456 ± 9	39 ± 1	1.9 ± 0.2	1.6 ± 0.2	168 ± 3	1050 ± 40	1077 ± 8	22.9 ± 0.2
2-Hydroxycarbamazepine	<LOQ	50 ± 1	6.1 ± 0.8	<LOQ	<LOQ	16 ± 1	164 ± 8	166 ± 2	<LOQ
3-Hydroxycarbamazepine	<LOQ	46 ± 2	3.6 ± 0.7	<LOQ	<LOQ	11.5 ± 0.7	143 ± 5	147 ± 2	<LOQ
10.11-dihydroxy-10.11-dihydro carbamazepine	<LOQ	690 ± 20	80 ± 3	2.6 ± 1	2.5 ± 0.3	264 ± 6	1930 ± 70	1950 ± 20	41 ± 3
Acridone	<LOQ	6.54 ± 0.06	0.6 ± 0.1	<LOQ	<LOQ	0.7 ± 0.2	4 ± 0.4	3.6 ± 0.3	<LOQ
10-hydroxy-10.11-dihydroxy carbamazepine	<LOQ	330 ± 20	22.4 ± 0.7	1.1 ± 0.3	0.9 ± 0.9	30.8 ± 0.9	265 ± 7	274 ± 1	<LOQ
Cetirizine	<LOQ	111 ± 3	21 ± 2	1.0 ± 0.6	0.6 ± 0.3	48 ± 8	240 ± 10	258 ± 4	6.2 ± 0.6
Cetirizine-N-Oxide	<LOQ	<LOQ	4 ± 1	2 ± 2	1.8 ± 0.5	10 ± 6	3 ± 2	4 ± 2	<LOQ
Citalopram	<LOQ	33 ± 1	4 ± 0.7	<LOQ	<LOQ	6.6 ± 0.1	100 ± 4	106 ± 3	<LOQ
Citalopram-N-Oxide	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Desmethylcitalopram	<LOQ	20 ± 1	2.6 ± 0.2	<LOQ	<LOQ	4 ± 0.2	53 ± 3	56 ± 0.3	<LOQ
Didemethylcitalopram	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Clarithromycin	0.4 ± 0.2	15.1 ± 0.8	11.6 ± 0.8	<LOQ	<LOQ	28 ± 1	230 ± 5	234 ± 6	8 ± 0.9
lopidogrel	<LOQ	7.48 ± 0.08	1.97 ± 0.1	<LOQ	<LOQ	4 ± 0.2	14.2 ± 0.4	13.9 ± 0.3	1.26 ± 0.06
Clopidogrel carboxylic acid	<LOQ	108 ± 1	19.1 ± 0.7	<LOQ	<LOQ	32 ± 1	226 ± 5	225 ± 7	7.7 ± 0.2
Diclofenac	<LOQ	730 ± 20	110 ± 3	<LOQ	<LOQ	281 ± 4	2470 ± 90	2535 ± 2	72 ± 4
4’-hydroxy-diclofenac	n.a.	n.a.	20 ± 1	<LOQ	<LOQ	47 ± 1	730 ± 20	882 ± 3	10.3 ± 0.7

Analyte	Concentration [ng/L]								
	Lake Stechlin	Teltow Canal	Saar (Rehlingen)	Nidda N1	Nidda N2	Mullbach before WWTP	Mullbach WWTP	Mullbach after WWTP	Rhine (Koblenz)
Diclofenac carboxylic acid	0.7 ± 0.4	22.2 ± 0.6	<LOQ	<LOQ	<LOQ	<LOQ	21.4 ± 0.8	22.4 ± 0.2	<LOQ
Diclofenac lactam	<LOQ	13.3 ± 0.5	<LOQ	<LOQ	<LOQ	4.4 ± 0.2	16.1 ± 0.6	16.5 ± 0.4	<LOQ
Diphenhydramine	2 ± 1	29 ± 2	2.1 ± 0.6	<LOQ	<LOQ	6 ± 1	75 ± 4	78 ± 3	1.4 ± 0.6
Diphenhydramine N-oxide	0.3 ± 0.6	1.78 ± 0.1	<LOQ	<LOQ	<LOQ	2 ± 1	2 ± 0.2	1.7 ± 0.2	<LOQ
N-Desmethyl diphenhydramin	<LOQ	6.8 ± 0.1	<LOQ	<LOQ	<LOQ	<LOQ	14.4 ± 0.4	16 ± 1	<LOQ
Duloxetine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Enalapril	<LOQ	<LOQ	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Enalaprilat	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	<LOQ
Fexofenadine	<LOQ	215 ± 7	6.3 ± 0.2	<LOQ	<LOQ	18.6 ± 0.9	122 ± 8	124 ± 1	43.1 ± 0.4
Flecainide-a	<LOQ	85 ± 2	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Flecainide-meta-O-dealkylated	<LOQ	9.7 ± 0.4	<LOQ	<LOQ	<LOQ	0.27 ± 0.01	4.45 ± 0.2	4.86 ± 0.02	<LOQ
Fluconazole	<LOQ	61 ± 3	5.4 ± 0.2	<LOQ	<LOQ	4.75 ± 0.08	63 ± 4	64 ± 3	<LOQ
Fluoxetine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Norfluoxetine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Furosemide	<LOQ	120 ± 4	<LOQ	<LOQ	<LOQ	<LOQ	223 ± 10	220 ± 10	<LOQ
Hydrochlorothiazide	<LOQ	1250 ± 60	170 ± 10	9 ± 1	8.6 ± 0.3	580 ± 20	5400 ± 300	5480 ± 80	96 ± 1
Chlorothiazide	<LOQ	61 ± 2	7 ± 1	<LOQ	<LOQ	32 ± 2	207 ± 7	213 ± 2	4.2 ± 0.6
Ibuprofen	<LOQ	<LOQ	46 ± 3	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	18 ± 6
2-hydroxy-Ibuprofen	<LOQ	<LOQ	74 ± 5	<LOQ	<LOQ	93 ± 5	<LOQ	<LOQ	<LOQ
Ibuprofe carboxylate	<LOQ	< LOQ	87 ± 9	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	54 ± 3
Irbesartan	<LOQ	324 ± 6	58 ± 3	1.2 ± 0.1	1 ± 0.2	143 ± 4	870 ± 40	900 ± 20	32 ± 1
Lamotrigine	<LOQ	740 ± 20	48 ± 3	<LOQ	<LOQ	150 ± 20	830 ± 10	780 ± 20	36 ± 2
Levetiracetam	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	10 ± 30	<LOQ	<LOQ	<LOQ

Analyte	Concentration [ng/L]								
	Lake Stechlin	Teltow Canal	Saar (Rehlingen)	Nidda N1	Nidda N2	Mullbach before WWTP	Mullbach WWTP	Mullbach after WWTP	Rhine (Koblenz)
Levetiracetam acid	<LOQ	360 ± 30	<LOQ	<LOQ	<LOQ	<LOQ	106 ± 4	99 ± 8	<LOQ
Lidocaine	<LOQ	202 ± 4	8.4 ± 0.4	<LOQ	<LOQ	35.4 ± 0.2	163 ± 5	168 ± 4	8.3 ± 0.6
Norlidocaine	<LOQ	37 ± 7	<LOQ	<LOQ	<LOQ	<LOQ	16.6 ± 0.7	17 ± 2	<LOQ
Metoprolol	<LOQ	387 ± 4	38.9 ± 0.5	<LOQ	<LOQ	44 ± 3	380 ± 6	370 ± 20	22.4 ± 0.6
Hydroxy metoprolol	<LOQ	38 ± 3	<LOQ	<LOQ	<LOQ	<LOQ	71 ± 5	74 ± 2	<LOQ
O-Desmethyl Metoprolol	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Naproxen	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	160 ± 20	155 ± 9	<LOQ
O-Desmethyl-Naproxen	<LOQ	29 ± 2	<LOQ	<LOQ	<LOQ	<LOQ	150 ± 10	190 ± 10	<LOQ
Olmesartan	<LOQ	241 ± 7	20.2 ± 0.6	<LOQ	<LOQ	34 ± 2	270 ± 10	277 ± 2	4.4 ± 0.9
Oxazepam	<LOQ	31.8 ± 0.5	15.4 ± 0.6	<LOQ	<LOQ	<LOQ	112 ± 5	114 ± 3	<LOQ
Phenytoin	<LOQ	9.6 ± 0.6	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Pregabalin	<LOQ	510 ± 30	45 ± 3	<LOQ	<LOQ	32 ± 5	320 ± 10	370 ± 20	<LOQ
Primidone	<LOQ	195 ± 7	15.2 ± 0.8	<LOQ	<LOQ	70 ± 3	360 ± 20	346 ± 9	<LOQ
Quetiapine	9 ± 6	2.1 ± 0.2	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	<LOQ
7-hydroxy-quetiapine	10 ± 6	27 ± 1	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	<LOQ
Quetipine sulfoxide	<LOQ	<LOQ	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	<LOQ
Ritalinic acid	<LOQ	58.4 ± 0.2	<LOQ	<LOQ	<LOQ	15.2 ± 0.4	85 ± 4	85 ± 3	<LOQ
Roxithromycin	n.a.	n.a.	3.2 ± 0.3	<LOQ	<LOQ	14 ± 2	97 ± 3	98 ± 6	n.a.
Sertraline	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
N-Desmethyl sertraline	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Sertraline ketone	n.a.	n.a.	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Sildenafil	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
N-Desmethyl Sildenafil	n.a.	n.a.	<LOQ	<LOQ	<LOQ	<LOQ	30 ± 10	<LOQ	<LOQ

Analyte	Concentration [ng/L]								
	Lake Stechlin	Teltow Canal	Saar (Rehlingen)	Nidda N1	Nidda N2	Mullbach before WWTP	Mullbach WWTP	Mullbach after WWTP	Rhine (Koblenz)
Sitagliptin	< LOQ	1430 ± 20	123 ± 5	4.5 ± 0.3	4.1 ± 0.3	183 ± 4	2170 ± 60	2220 ± 20	64.8 ± 0.4
Sotalol	<LOQ	61 ± 2	34 ± 2	<LOQ	<LOQ	22 ± 3	117 ± 4	107 ± 6	<LOQ
Sulfamethoxazole	<LOQ	200 ± 4	13.6 ± 0.8	<LOQ	<LOQ	12 ± 0.8	185 ± 9	185 ± 5	14 ± 1
N-Acetyl sulfamethoxazole	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Sulpiride	<LOQ	89 ± 3	8.3 ± 0.4	<LOQ	<LOQ	28 ± 1	135 ± 2	136 ± 7	3 ± 1
Tadalafil	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Telmisartan	6 ± 3	295 ± 2	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	27 ± 3
Torasemide	<LOQ	82 ± 2	7.4 ± 0.4	<LOQ	<LOQ	22.1 ± 0.4	190 ± 10	192 ± 7	<LOQ
Hydroxytorasemide	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Tramadol	<LOQ	406 ± 6	42 ± 2	0.9 ± 0.5	1.05 ± 0.09	72 ± 2	420 ± 20	428 ± 4	20 ± 1
Dehydrotramadol	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
O-Desmethyl-Tramadol	<LOQ	310 ± 10	60.7 ± 0.9	<LOQ	<LOQ	96 ± 2	690 ± 20	680 ± 50	21.8 ± 0.9
N-Desmethyl-Tramadol	<LOQ	90 ± 4	5.3 ± 1	<LOQ	<LOQ	19 ± 2	131 ± 8	136 ± 7	4.9 ± 0.8
N,O-Didesmethyl-Tramadol	<LOQ	480 ± 10	38 ± 2	<LOQ	<LOQ	69 ± 2	460 ± 20	480 ± 10	16 ± 2
Tramadol-N-oxide	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Trimethoprim	<LOQ	34 ± 2	4.6 ± 0.8	<LOQ	<LOQ	6 ± 1	136 ± 2	138 ± 7	3.3 ± 0.4
3-Desmethyl trimethoprim	<LOQ	5.4 ± 0.6	<LOQ	<LOQ	<LOQ	<LOQ	27.3 ± 0.4	29 ± 2	<LOQ
5-(3.4.5-Trimethoxybenzoyl)-2.4-pyrimidinediamine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Valsartan	<LOQ	447 ± 8	178 ± 3	6.4 ± 0.3	7.6 ± 0.4	95 ± 2	109 ± 3	117 ± 1	142 ± 3
4-Hydroxy-Valsartan-a	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	n.a.
Valsartanic acid	<LOQ	4580 ± 80	177 ± 6	<LOQ	<LOQ	800 ± 30	5700 ± 200	5700 ± 400	100 ± 20
Venlafaxine	< LOQ	179.3 ± 0.6	19 ± 1	<LOQ	<LOQ	61 ± 2	330 ± 10	329 ± 6	16.6 ± 0.4

Analyte	Concentration [ng/L]								
	Lake Stechlin	Teltow Canal	Saar (Rehlingen)	Nidda N1	Nidda N2	Mullbach before WWTP	Mullbach WWTP	Mullbach after WWTP	Rhine (Koblenz)
N-Desmethyl venlafaxine	<LOQ	30.9 ± 0.6	<LOQ	<LOQ	<LOQ	<LOQ	54 ± 3	56 ± 1	<LOQ
O-Desmethyl venlafaxine	<LOQ	530 ± 30	48 ± 4	<LOQ	<LOQ	188 ± 4	1140 ± 20	1100 ± 30	45 ± 4
N,O-Desmethyl venlafaxine	<LOQ	98 ± 2	5.9 ± 0.2	<LOQ	<LOQ	<LOQ	128 ± 1	129.7 ± 0.4	<LOQ
Venlafaxine N-oxide	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Xipamide	<LOQ	6.4 ± 0.1	3.25 ± 0.09	<LOQ	<LOQ	<LOQ	20 ± 1	21.5 ± 0.2	<LOQ

Table 17: Concentrations of the hormonal acting pharmaceuticals (ng/L)

Substance	Lake Stechlin	Teltow Canal	River Saar	River Nidda N1	River Nidda N2	Muehlbach upstream WWTP	Muehlbach WWTP	Muehlbach downstream WWTP
Canrenone	<LOQ	2.9	0.7	<LOQ	<LOQ	1.0	11	11
11α-Hydroxy canrenone	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.6	1.5
7α-Thiomethyl spironolac-tone	<LOQ	0.6	0.1	<LOQ	<LOQ	0.1	0.8	0.8
Beclomethasone	<LOQ	0.07	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.05
Betamethasone	<LOQ	1.0	0.05	<LOQ	<LOQ	0.1	0.5	0.8
-valerate	<LOQ	0.2	0.1	<LOQ	<LOQ	0.2	2.1	1.5
-propionate	<LOQ	0.4	<LOQ	<LOQ	<LOQ	<LOQ	1.2	1.0
6α-Methylprednisolone	<LOQ	0.2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.07

Substance	Lake Stechlin	Teltow Canal	River Saar	River Nidda N1	River Nidda N2	Muehlbach upstream WWTP	Muehlbach WWTP	Muehlbach downstream WWTP
6α-Methylprednisolone propionate	<LOQ	0.9	<LOQ	<LOQ	<LOQ	0.1	1.2	0.8
Triamcinolone acetonide	<LOQ	7.6	0.6	<LOQ	<LOQ	1.0	7.2	6.8
6ß-Hydroxy triamcinolone acetonide	<LOQ	1.2	0.08	<LOQ	<LOQ	0.1	1.1	1.0
Fluticasone 17-propionate	<LOQ	0.3	<LOQ	<LOQ	<LOQ	<LOQ	0.1	0.1
Fluticasone 17-furoate	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.06
Mometasone 17-furoate	<LOQ	0.2	<LOQ	<LOQ	<LOQ	<LOQ	0.9	0.6
Fluocinolone acetonide	<LOQ	0.09	<LOQ	<LOQ	<LOQ	<LOQ	0.2	0.2
Clobetasol propionate	0.05	1.7	0.08	<LOQ	<LOQ	0.1	0.9	0.8
Budesonide	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.7	0.7
Prednisolone	0.05	<LOQ	<LOQ	0.06	0.05	0.1	0.1	0.1
Prednisone	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.2	0.2
Hydrocortisone	0.2	0.2	0.8	0.2	0.2	0.4	1.8	1.6
Cortisone	0.1	0.08	0.4	0.1	0.2	0.09	0.3	0.2
Cyproterone acetate	<LOQ	0.9	<LOQ	<LOQ	<LOQ	<LOQ	0.5	0.4
Medroxyprogesterone acetate	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.2	0.2	0.2
17α-Hydroxyprogesterone	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.7	0.7
Estrone	0.2	0.3	1.3	0.3	0.3	12	3.5	2.8

4.3 Comparison of suspect screening and target analysis

Suspect screening and target analysis are totally different approaches in environmental monitoring. Both have benefits and limitations and give different insights into the pollution of a water system. While suspect screening give an overall overview over all compounds in the water target methods only measure a limited number of selected analytes. Therefore LODs of these methods are much smaller and quantitative results are determinable. Both kinds of methods were used for analysis of water samples from the river Rhine and the Teltow Canal. Suspect screening find 97 and 24 pharmaceuticals in the Teltow Canal and river Rhine, respectively. By target analysis 107 and 63 pharmaceuticals were found above the LOD in the Teltow Canal and the river Rhine, respectively. Figure 21 illustrate the differences and compliances of the two approaches. Higher detection rates of the target analysis can be explained by the included hormonal pharmaceuticals which can only be detected after an extensive enrichment and clean up using an extreme sensitive method. The same is true for the extreme polar pharmaceuticals which can't be analyzed by the used suspect screening method. On the other hand several pharmaceuticals and metabolites which were not included into the target analysis methods could be found in the Teltow Canal by suspect screening (ref. Table 18). These were during prioritization not considered pharmaceuticals like the anticholinergic trospium. But also metabolites and transformation products of parent pharmaceuticals which were included in the target analysis could found with high peak intensities. This demonstrates the necessity of analysis of metabolites and transformation products for acquisition of the complete burden of a water system and calculation of mass balances.

Figure 21: Comparison of the developed target analytical methods and the suspect screening approach exemplarily for the Teltow Canal (strongly wastewater affected) and river Rhine (slightly wastewater affected). (Source: Own representation, Federal Institute of Hydrology)

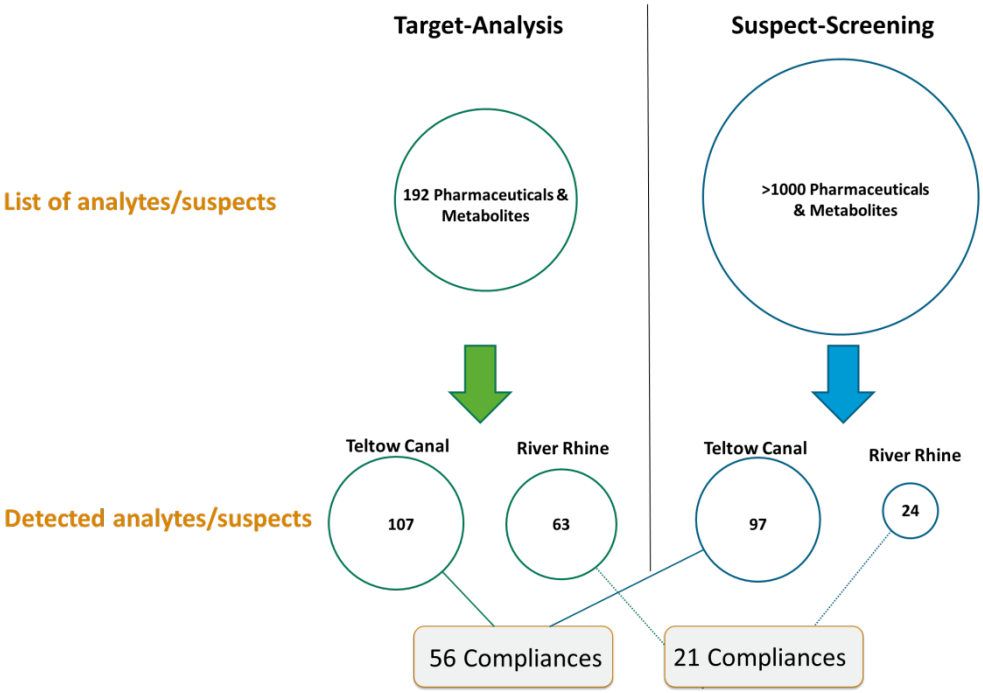


Table 18: Selected compounds detected only by suspect screening

Name	Teltow Canal	River Rhine
14-Hydroxy clarithromycine	✓	×
Hydroxy norlidocaine	✓	✓
4-Aminoantipyrin	✓	×
9-Carboxylic acid-acridine	✓	×
9-Carboxymethoxymethylguanine	✓	×
Carboxy Torasemide	✓	✓
Celecoxib carboxylic acid	✓	×
Desmethyl tilidine	✓	✓
Didesmethyl tilidine	✓	×
EDDP (2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidene)	✓	×
Amantadine	✓	×
Amitriptyline	✓	×
Atazanavir	✓	×
Celiprolol	✓	×
Clozapine	✓	×
Levorphanol	✓	×
Methadone	✓	×
Tilidine	✓	×
Trospium	✓	×
Xylometaxoline	✓	×

✓=detected, × = not detected

5 Summary and elaboration of recommendations (Work package 4):

The ubiquity occurrence of pharmaceuticals and their metabolites and transformation products could be clearly shown in this project. These results underline the urgency of the environmental pollution of water systems by pharmaceuticals. Especially concerned are water systems affect by treated wastewater like the Teltow Canal. Using a suspect screening method 97 pharmaceutical and metabolites/transformation products could be detected. In the river Rhine, which is lesser affected by treated wastewater, 24 compounds were detected by this method. A wide range of pharmaceuticals was detected pointing out that pharmaceuticals are urgent contaminates in surface waters and the necessity of a broad monitoring approach.

For quantification of high priority pharmaceuticals target methods were developed. Despite the large polarity range of pharmaceuticals one multi-method is not recommended. For analysis of extreme polar pharmaceuticals HILIC is the method of choice. For sample preparation freeze drying gave the best results enabling pre-concentration of anionic, cationic, zwitterionic and neutral analytes at the same time. In this project benefits and limitations of this technic are highlighted and capability for environmental monitoring is demonstrated [122]. But further optimization and evaluations are necessary before this method is suitable for routine analysis and its application as standard method. From the group of extreme polar pharmaceuticals oxipurinol, guanyl urea and diatrozoate were found in highest concentrations.

For the middle polar pharmaceuticals a RP-HPLC method using direct sample injection without sample pretreatment is recommended. This procedure avoids discrimination of analytes by different extraction efficiencies in for example solid phase extraction or other sample pretreatment steps. During monitoring campaign candesartan, carbamazepine and its metabolites, hydrochlorothiazide, sitagliptin and valsartanic acid show the highest detection rates and concentrations. These are pharmaceuticals for treatment of high blood pressure, diabetes and mental diseases. They are long term therapeutics with high consumption amounts. WWTPs seems to be the main sources.

Concentrations of steroid hormones in surface waters lay in the ng/L range, so an analytical method with extreme low limits of quantification (<0.5 ng/L) is needed. Thus low LOQs can be realized by large volume solid phase extraction, a silica clean-up and a high performance LC-MS-MS method. Almost all analyzed hormonal pharmaceuticals could be detected in waters with high affection with treated waste water. Highest concentrations were found for triamcinolone acetonide and canrenone.

Beside the widespread occurrence of the analyzed pharmaceuticals in surface water the results reveal that for some pharmaceuticals metabolites or transformation products show higher concentrations than the initial compounds. This is the case for example allopurinol and its metabolite oxipurinol or tramadol and its metabolites O-desmethyltramadol and N,O-didesmethyltramadol. In thus cases a monitoring of the metabolites instead of the original pharmaceutical seems to be reasonable. Meaning of different metabolites and transformation products and their suitability for environmental monitoring will be a major part in a follow-up project. Concerning the hormonal active pharmaceuticals the study clearly demonstrates the necessity of consideration of the different ester derivatives of the steroid hormones for environmental monitoring. Stability of the different esters and formation of transformation products in the environment and during wastewater treatment will be investigated within a follow-up project.

Further project challenges

Unfortunately no results for sediment/suspended particular matter and biota could be generated within this project. Due to the complexity of theses matrices an elaborated sample preparation and

clean-up is necessary. The basics for these methods were implemented and optimization and validation will be done within a follow-up project. Within the follow-up project a comprehensive monitoring campaign including water, suspended particular matter and biota samples from all five sampling sites will be executed. Additionally a retrospective time trend analysis in particular matter and biota samples from the German environmental specimen bank will be carried out. Partitioning of the pharmaceuticals between the compartments water, sediment/particular matter and biota will be derived from the analysis data. These results will be the basis for the elaboration of recommendations for the analysis of pharmaceuticals in environmental samples.

Project publications

The results of the project were published in peer-reviewed scientific journals:

- Utilization of large volume zwitterionic hydrophilic interaction liquid chromatography for the analysis of polar pharmaceuticals in aqueous environmental samples: Benefits and limitations [122].
- Occurrence of Glucocorticoids, Mineralocorticoids, and Progestogens in Various Treated Wastewater, Rivers, and Streams [123].

Furthermore the results were presented in form of oral lecture and poster presentations at scientific conferences:

- Utilization of large volume HILIC for the analysis of pharmaceuticals in aqueous environmental: benefits and limitations. Lise Boulard, Georg Dierkes, Thomas Ternes oral lecture at GDCh Workshop: Hoch polare Stoffe:Analytik, Auftreten, Quellen und Wirkungen, 2017
- Utilization of large volume HILIC for the analysis of pharmaceuticals in aqueous environmental: benefits and limitations. Lise Boulard, Georg Dierkes, Thomas Ternes, poster at Wassertagung, 2018.
- Steroidhormone in der Umwelt: Vorkommen von Progestagenen, Glucocorticoiden und Mineralocorticoiden in Kläranlagenabläufen und Fließgewässern. Weizel, A. Schlüsener, M. Dierkes, G., Ternes, T.A. oral lecture at Wassertagung, 2018
- Traget Analysis of a Large Number of Steroid Hormones: Corticosteroids and Progesogens in Wastewater and Receiving Surface Waters. Authors: Weizel, A. Schlüsener, M. Dierkes, G., Ternes, T.A. oral lecture at Water JPI Conference 2018

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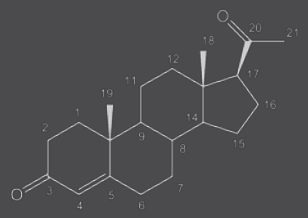
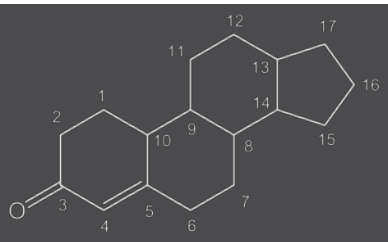
7 Appendix

Table A 1: Abbreviations and chemical structures of target steroid hormones.



Basic structure of Gluco- and Mineralocorticoids



Basic structure of Progestogens

			Position					
Abbreviation	**Name**	**1 + 2**	**6**	**9**	**11**	**16**	**17**	**21**
Glucocorticoids								
BDN-m1	6ß-Hydroxy budesonide	C=C	ß-OH		-OH	-O-HC(C_3H_7)-O-		-OH
DMS-m1	6ß-Hydroxy dexamethasone	C=C	ß-OH	-F	-OH	α-CH_3	-OH	-OH
TRIact-m1	6ß-Triamcinolone acetonide	C=C	ß-OH	-F	-OH	-O-C(CH_3)$_2$-O-		-OH
MPNL	6α-Methylprednisolone	C=C	α-CH_3		-OH		-OH	-OH
MPNLprop	6α-Methylprednisolone 21-propionate	C=C	α-CH_3		-OH		-OH	-O-COC_2H_5
MPNLacp	6α-Methylprednisolone aceponate	C=C	α-CH_3		-OH		-O-COC_2H_5	-O-$COCH_3$
BEC	Beclomethasone	C=C		-Cl	-OH	ß-CH_3	-OH	-OH
BECprop	Beclomethasone 17-propionate	C=C		-Cl	-OH	ß-CH_3	-O-COC_2H_5	-OH
BECdiprop	Beclomethasone dipropionate	C=C		-Cl	-OH	ß-CH_3	-O-COC_2H_5	-O-COC_2H_5
BMS	Betamethasone	C=C		-F	-OH	ß-CH_3	-OH	-OH
BMSprop	Betamethasone 17-propionate	C=C		-F	-OH	ß-CH_3	-O-COC_2H_5	-OH
BMSval	Betamethasone 17-valerate	C=C		-F	-OH	ß-CH_3	-O-COC_4H_9	-OH
BMSac	Betamethasone 21-acetate	C=C		-F	-OH	ß-CH_3	-OH	-O-$COCH_3$
BMSdiprop	Betamethasone dipropionate	C=C		-F	-OH	ß-CH_3	-O-COC_2H_5	-O-COC_2H_5
BDN	Budesonide	C=C			-OH	-O-HC(C_3H_7)-O-		-OH
CIC	Ciclesonide	C=C			-OH	-O-HC(C_6H_{11})-O-		-O-$COCH(CH_3)_2$
CLO	Clobetasol	C=C		-F	-OH	ß-CH_3	-OH	-Cl
CLOprop	Clobetasol propionate	C=C		-F	-OH	ß-CH_3	-O-COC_2H_5	-Cl
HCOR	Cortisol				-OH		-OH	-OH
COR	Cortisone				=O		-OH	-OH
CIC-m1	Desisobuturyl ciclesonide	C=C			-OH	-O-HC(C_6H_{11})-O-		-OH
DMS	Dexamethasone	C=C		-F	-OH	α-CH_3	-OH	-OH
DMSac	Dexamethasone 21-acetate	C=C		-F	-OH	α-CH_3	-OH	-O-$COCH_3$
DFCval	Diflucortolone valerate	C=C	-F	-F	-OH	α-CH_3		-O-COC_4H_9
FMS	Flumethasone	C=C	-F	-F	-OH	ß-CH_3	-OH	-OH
FMSpiv	Flumethasone pivalate	C=C	-F	-F	-OH	ß-CH_3	-OH	-O-$COC(CH_3)_3$
FCNact	Fluocinolone acetonide	C=C	-F	-F	-OH	-O-C(CH_3)$_2$-O-		-OH
FML	Fluorometholone	C=C	α-CH_3	-F	-OH		-OH	

FLUfur	Fluticasone furoate	C=C	-F	-F	-OH	α-CH_3	-O-COC_4H_3O	SCH_2F [a]
FLUprop	Fluticasone propionate	C=C	-F	-F	-OH	α-CH_3	-O-COC_2H_5	SCH_2F [a]
HAL	Halcinonide			-F	-OH	-O-$C(CH_3)_2$-O-		-Cl
HLM	Halometasone	C(1)=C(2)-Cl	-F	-F	-OH	α-CH_3	-OH	-OH
MOM	Mometasone	C=C		-Cl	-OH	α-CH_3	-OH	-Cl
MOMfur	Mometasone furoate	C=C		-Cl	-OH	α-CH_3	-O-COC_4H_3O	-Cl
PNL	Prednisolone	C=C			-OH		-OH	-OH
PNS	Prednisone	C=C			=O		-OH	-OH
TRIact	Triamcinolone acetonide	C=C		-F	-OH	-O-$C(CH_3)_2$-O-		-OH

		Position						
Abbre-viation	**Name**	**1 + 2**	**7**	**9**	**11**	**16**	**17**	**21**

Mineralocorticoids

CAN-m1	11α-Hydroxy canrenone		C(6)=C(7)		-OH		-O-COC_2H_4- [b]	
SPL-m1	7α-Thiomethyl spironolactone		α-S-CH_3				-O-COC_2H_4- [b]	
CAN	Canrenone		C(6)=C(7)				-O-COC_2H_4- [b]	
FLC	Fludrocortisone			-F	-OH	-OH	-OH	-OH
FLCac	Fludrocortisone acetate			-F	-OH	-OH	-OH	-O-$COCH_3$

		Position						
Abbre-viation	**Name**	**1 + 2**	**6**	**10**	**11**	**13**	**16**	**17**

Progestogens

HPG	17α-Hydroxy progester-one			ß-CH_3		ß-CH_3		α-OH, -$COCH_3$
DIE-m1	6ß-Hydroxy dienogest		-ß-OH	C(9)=C(10)				-OH, -CH_2-CN
MRPac-m1	6ß-Hydroxy medroxy progesterone acetate		α-CH_3	ß-CH_3, α-OH		ß-CH_3		α-O-$COCH_3$,-$COCH_3$
CLM	Chlormadinone		Cl-C(6)=C(7)	ß-CH_3		ß-CH_3		-$COCH_3$, -OH
CLMac	Chlormadinone acetate		Cl-C(6)=C(7)	ß-CH_3		ß-CH_3		-$COCH_3$, -O-$COCH_3$
CYP	Cyproterone	-CH_2-	Cl-C(6)=C(7)	ß-CH_3		ß-CH_3		-$COCH_3$, -OH
CYPac	Cyproterone acetate	-CH_2-	Cl-C(6)=C(7)	ß-CH_3		ß-CH_3		-$COCH_3$, -O-$COCH_3$
DIE	Dienogest			C(9)=C(10)				-OH, -CH_2-CN
DPN	Drospirenone		(6)-CH_2-(7)				(15)-CH_2-(16)	-O-COC_2H_4-
ETG	Etonogestrel			ß-CH_3	$=CH_2$	ß-C_2H_5		-CCH, -OH
GES	Gestodene					ß-C_2H_5	(15)-CH_2-(16)	-CCH, -OH
LNG	Levonorgestrel					ß-C_2H_5		-CCH, ß-OH
MRP	Medroxy progesterone		α-CH_3	ß-CH_3		ß-CH_3		α-OH,-$COCH_3$
MRPac	Medroxy progesterone acetate		α-CH_3	ß-CH_3		ß-CH_3		α-O-$COCH_3$,-$COCH_3$
MEG	Megestrol		CH_3-C(6)=C(7)	ß-CH_3		ß-CH_3		α-OH, -$COCH_3$
MEGac	Megestrol acetate		CH_3-C(6)=C(7)	ß-CH_3		ß-CH_3		α-O-$COCH_3$,-$COCH_3$
NES	Norethisterone					ß-CH_3		-CCH, ß-OH
NESac	Norethisterone acetate					ß-CH_3		-CCH, ß-O-$COCH_3$

[a] sulfur instead of C(21)
[b] without -$COCH_3$ group at pos. C17

Table A 2: Results of the suspect-screening/ non-target approach.

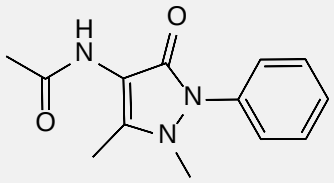
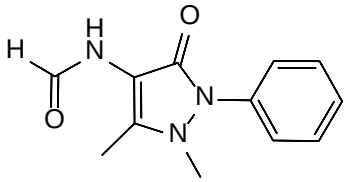
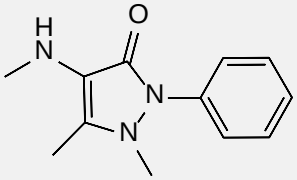
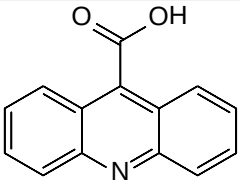
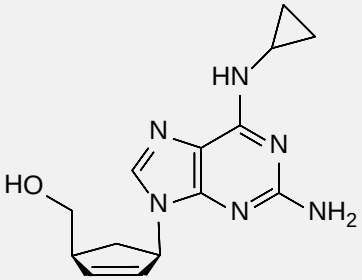
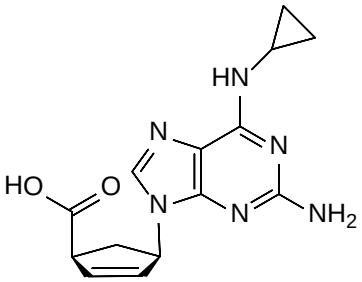
Name	Chem. Formula	Teltow Canal	River Rhine
10, 11-Dihydro-10-hydroxy-carbamazepine	C15H14N2O2	✓	×
14-Hydroxy clarithromycin	C38H69NO14	✓	×
Hydroxy norlidocaine	C12H18N2O2	✓	✓
4-Acetylaminoantipyrine	C13H15N3O2	✓	✓
4-Aminoantipyrin	C11H13N3O	✓	×
4-Formylaminoantipyrin	C12H13N3O2	✓	✓
9-Carboxylic acid-acridine	C14H9NO2	✓	×
9-Carboxymethoxymethylguanine	C8H9N5O4	✓	×
Acridone	C13H9NO	✓	×
Aliskiren	C30H53N3O6	✓	×
Amantadine	C10H17N	✓	×
Aminofurantoin	C8H8N4O3	✓	×
Amisulpride	C17H27N3O4S	✓	✓
Amitriptyline	C20H23N	✓	×
Atazanavir	C38H52N6O7	✓	×
Atenolol	C14H22N2O3	✓	×
Atenolol Acid	C14H21NO4	✓	✓
Azithromycin	C38H72N2O12	✓	×
Bezafibrate	C19H20ClNO4	✓	×
Bicalutamide	C18H14F4N2O4S	✓	×
Bisoprolol	C18H31NO4	✓	×
Bisoprolol TP	C13H19NO4	✓	✓
Candesartan	C24H20N6O3	✓	✓
Carbamazepine	C15H12N2O	✓	✓
Carboxy Torasemide	C16H18N4O5S	✓	×
Celecoxib carboxylic acid	C17H12F3N3O4S	✓	×
Celiprolol	C20H33N3O4	✓	×
Cetirizine	C21H25ClN2O3	✓	×
Citalopram	C20H21FN2O	✓	×
Clindamycine	C18H33ClN2O5S	✓	×
Clindamycine sulfoxide	C18H33ClN2O6S	✓	×
Clopidogrel	C16H16ClNO2S	✓	×
Clozapine	C18H19ClN4	✓	×
DEET	C12H17NO	✓	✓

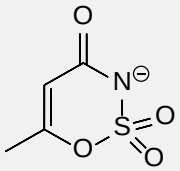
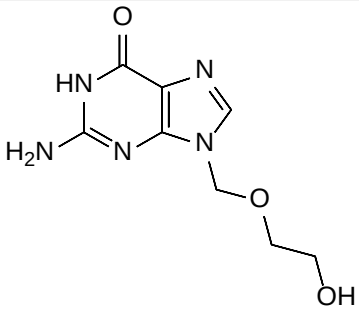
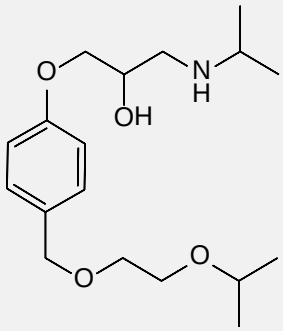
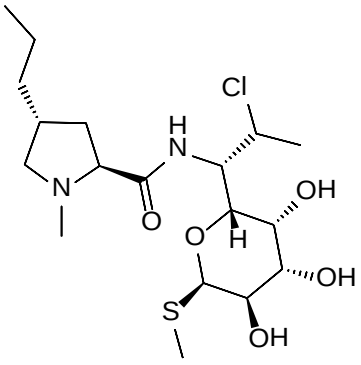
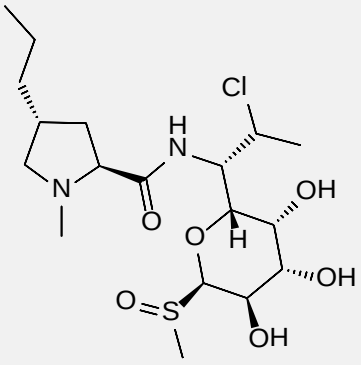
Name	Chem. Formula	Teltow Canal	River Rhine
Desmethyl tilidine	C16H21NO2	✓	✓
Diatrizoate	C11H9I3N2O4	✓	×
Diclofenac	C14H11Cl2NO2	✓	×
Diclofenac-Lactam	C14H9Cl2NO	✓	×
Didesmethyl tilidine	C15H19NO2	✓	×
Didesmethyl venlafaxine	C15H23NO2	✓	×
Diphenhydramine	C17H21NO	✓	×
EDDP (2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidene)	C20H23N	✓	×
Erythromycin-H2O	C37H65NO12	✓	×
Fexofenadine	C32H39NO4	✓	✓
Flecainide	C17H20F6N2O3	✓	×
Furosemide	C12H11ClN2O5S	✓	×
Gabapentine	C9H17NO2	✓	✓
Gabapentine/Lactam	C9H15NO	✓	×
Hydrochlorothiazide	C7H8ClN3O4S2	✓	✓
Hydroxy metoprolol	C15H25NO4	✓	×
Iomeprol	C17H22I3N3O8	✓	✓
Iopamidol	C17H22I3N3O8	✓	×
Irbesartan	C25H28N6O	✓	✓
Irbesartan IB3a (Oxidation)	C25H26N6O2	✓	×
Irbesartan IB3b (Oxidation)	C25H26N6O2	✓	×
Lamotrigine	C9H7Cl2N5	✓	✓
Lamotrigine related compound C	C9H6Cl2N4O	✓	×
Lamotrigine-N2-glucoronide TP 430	C15H13Cl2N5O6	✓	✓
Levorphanol	C17H23NO	✓	×
Lidocaine	C14H22N2O	✓	×
Losartan	C22H23ClN6O	✓	×
Losartan Carboxylic Acid	C22H21ClN6O2	✓	×
Methadone	C21H27NO	✓	×
Metoprolol	C15H25NO3	✓	✓
N,N-didesmethyltramadol	C14H21NO2	✓	×
N,O-Didesmethyltramadol	C14H21NO2	✓	×
N,O-Didesmethylvenlafaxine	C15H23NO2	✓	×
N-Acetyl sitagliptin	C18H17F6N5O2	✓	×
N-Desmethyl clindamycine	C17H31ClNO5S	✓	×
N-Desmethyltramadol	C15H23NO2	✓	×
Norcitalopram	C19H19FN2O	✓	×

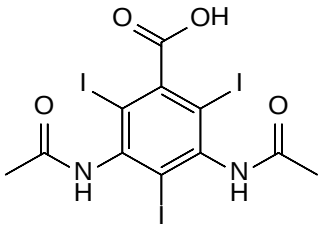
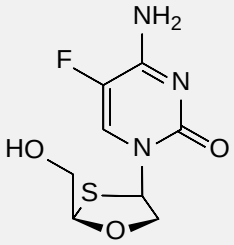
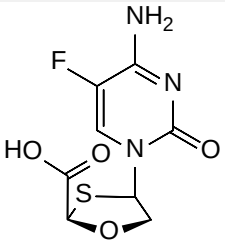
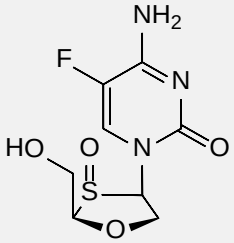
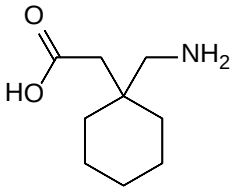
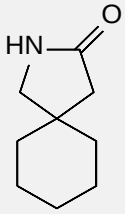
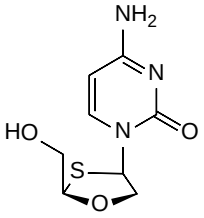
Name	Chem. Formula	Teltow Canal	River Rhine
Norlidocaine	C12H18N2O	✓	×
O-Desmethyltramadol	C15H23NO2	✓	×
O-Desmethylvenlafaxine	C16H25NO2	✓	×
Olmesartan	C24H26N6O3	✓	×
Oxazepam	C15H11ClN2O2	✓	×
Oxcabazepine	C15H12N2O2	✓	✓
Oxypurinol	C5H4N4O2	✓	×
Pregabaline	C8H17NO2	✓	×
Propranolol	C16H21NO2	✓	×
Ritalinic Acid	C13H17NO2	✓	×
Sitagliptin	C16H15F6N5O	✓	✓
Sulfamethoxazole	C10H11N3O3S	✓	✓
Sulfapyridine	C11H11N3O2S	✓	×
Sulpiride	C15H23N3O4S	✓	×
Telmisartan	C33H30N4O2	✓	×
Tilidine	C17H23NO2	✓	×
Torasemide	C16H20N4O3S	✓	×
Tramadol	C16H25NO2	✓	✓
Trimethoprim	C14H18N4O3	✓	×
Trospium	C25H30NO3	✓	×
Valsartan	C24H29N5O3	✓	×
Valsartan TP	C19H23N5O	✓	×
Valsartan acid	C14H10N4O2	✓	✓
Venlafaxine	C17H27NO2	✓	✓
Xylometazoline	C16H24N2	✓	×

×: not detected; ✓: identified

Table A 3: Structure of the analytes. (Source: Own representation, Federal Institute of Hydrology)

Name	CAS No	Structure
4-Acetamidoantipyrine	83-15-8	
4-Formylaminoantipyrine	1672-58-8	
4-Methylaminoantipyrine	519-98-2	
9-Acridine carboxylic acid	5336-90-3	
Abacavir	136470-78-5	
Abacavir carboxylate	384380-52-3	

Acesulfame	55589-62-3	
Acyclovir	59277-89-3	
Bisoprolol	66722-44-9	
Clindamycin	18323-44-9	
Clindamycin sulfoxide	22431-46-5	

Diatrizoate	737-31-5	
Emtricitabine	143491-57-0	
Emtricitabine carboxylate	1238210-10-0	
Emtricitabine *S*-oxide	152128-77-3	
Gabapentin	60142-96-3	
Gabapentin lactam	64744-50-9	
Lamivudine	134678-17-4	

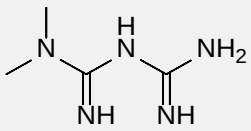
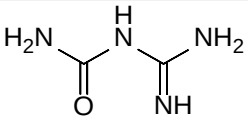
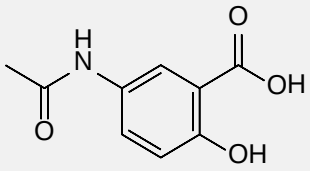
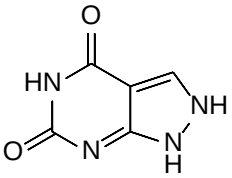
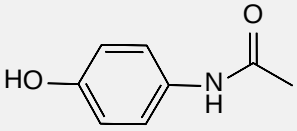
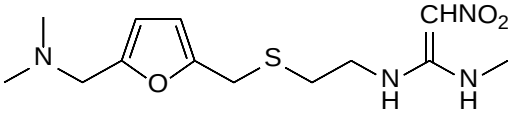
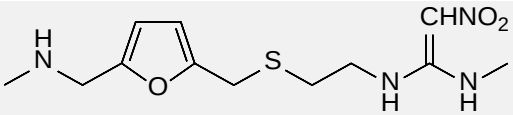
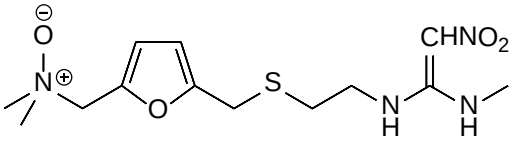
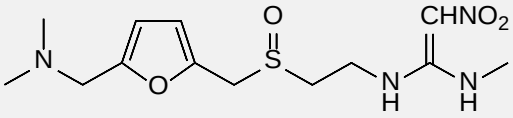
Metformin	657-24-9	
Guanyl urea	141-83-3	
N-acetyl mesalazine	51-59-2	
Oxipurinol	2465-59-0	
Paracetamol	103-90-2	
Ranitidine	66357-35-5	
Desmethyl ranitidine	66357-25-3	
Ranitidine *N*-oxide	73857-20-2	
Ranitidine *S*-oxide	73851-70-4	

Table A 4: Multiple reaction monitoring parameters of the analytes.

Name analytes	Retention time [min]	MRM 1 (Quantification)	MRM 2 (Confirmation)	DP	CE	CXP	Polarity
Analytes							
4-Acetamidoantipyrine	4.69	246.1/83	246.1/204	60	20/45	16/6	Positive
4-Formylaminoantipyrine	4.26	232.1/104	232.1/214	65	20/32	13/5	Positive
4-Methylaminoantipyrine	3.92	218.1/97	218.1/187	70	19/15	18/13	Positive
9-Acridine carboxylic acid	10.65	224.17/167	224.17/196.02	86	37/57	16/14	Positive
Abacavir	5.43	287.2/191	287.2/79	31	25/47	30/12	Positive
Abacavir carboxylate	6.99	299.1/189	299.1/132	-45	-48/-19	-9/-9	Negative
Acesulfame	3.41	161.8/82	161.8/78	-50	-38/-22	-3/-5	Negative
Acyclovir	10.81	226.1/152.1	226.1/135.1	71	17/43	12/14	Positive
Bisoprolol	8.96	326.2/116	326.2/74	76	27/41	10/6	Positive
Clindamycin	12.5	425.2/126	425.2/377	70	50/28	6/11	Positive
Clindamycin sulfoxide	14.61	441.2/377	441.2/126	55	26/41	11/6	Positive
Diatrizoate	14.28	614.8/233	614.8/361	91	79/42	4/10	Positive
Emtricitabine	4.68	248.1/130	248.1/113	61	19/53	10/10	Positive
Emtricitabine carboxylate	13.97	262/130	262/113	48	23/56	10/10	Positive
Emtricitabine *S*-oxide	7.4	264/130	264/113	60	27/57	10/10	Positive
Gabapentin	13.05	172.1/154.2	172.1/137.2	55	19/22	10/10	Positive
Gabapentin lactam	3.18	154.1/95	154.1/67	80	30/40	12/12	Positive
Lamivudine	6.95	230.1/112	230.1/95	56	19/51	18/6	Positive
Metformin	13.34	130.1/71	130.1/60	36	31/19	4/4	Positive
Guanyl urea	14.2	103.1/60	103.1/86	25	18/14	10/15	Positive
N-Acetyl mesalazine	6.86	194/107	194/150	-50	-29/-22	-6/-11	Negative
Oxipurinol	5.25	151/108	151/42	-70	-24/-32	-6/-5	Negative
Paracetamol	3.42	152.1/110.1	152.1/65	80	20/50	4/4	Positive
Ranitidine	12.75	315.1/176.2	315.1/130	30	25/36	10/10	Positive
Desmethyl ranitidine	13.22	301.1/124	301.1/176.2	50	20/35	10/10	Positive
Ranitidine *N*-oxide	13.75	331.1/176.2	331.1/124.3	30	25/20	10/10	Positive
Ranitidine *S*-oxide	16.63	331.1/138	331.1/188	45	25/18	10/10	Positive
Surrogates							
4-Acetamidoantipyrine-d_3	4.69	249.1/231.2	-	50	22	18	Positive
Abacavir-d_4	5.43	291.2/195	-	130	52	10	Positive
Acyclovir-d_4	10.81	230.1/152.1	-	46	19	12	Positive
Bisoprolol-d_7	8.96	333.3/123	-	90	26	6	Positive
Clindamycin-d_3	12.5	428.2/129	-	95	42	10	Positive
Emtricitabine-$^{13}C,^{15}N_2$	4.59	251/133	-	54	19	12	Positive
Gabapentin lactam-d_6	3.18	160.3/101.1	-	81	33	8	Positive
Lamivudine-$^{13}C,^{15}N_2$	6.95	233.1/115	-	95	20	9	Positive

Name analytes	Retention time [min]	MRM 1 (Quantification)	MRM 2 (Confirmation)	DP	CE	CXP	Polarity
Guanyl urea-$^{15}N_4$	14.2	107.1/63	-	40	17	10	Positive
Paracetamol-d_4	3.42	156.2/114	-	65	24	7	Positive
Oxipurinol-^{13}C,$^{15}N_2$	5.25	154/111	-	-65	-27	-8	Negative
Acesulfame-d_4	3.41	165.74/86.1	-	-50	-22	-5	Negative
Diatrizoate-d_6	14.28	620.9/367.1	-	92	25	6	Positive

Table A 5: Analytes with their corresponding internal standard.

Standard	Internal standard
4-Acetamidoantipyrine	4-Acetamidoantipyrine-d_3
4-Formylaminoantipyrine	4-Acetamidoantipyrine-d_3
4-Methylaminoantipyrine	n.a.
9-Acridine carboxylic acid	n.a.
Abacavir	Abacavir-d_4
Abacavir carboxylate	n.a.
Acesulfame	Acesulfame-d_4
Acyclovir	Acyclovir-d_4
Bisoprolol	Bisoprolol-d_7
Clindamycin	Clindamycin-d_3
Clindamycin sulfoxide	n.a.
Diatrizoate	Diatrizoate-d_6
Emtricitabine	Emtricitabine-$^{13}C,^{15}N_2$
Emtricitabine carboxylate	n.a.
Emtricitabine *S*-oxide	n.a.
Gabapentin	n.a.
Gabapentin lactam	Gabapentin lactam-d_6
Lamivudine	Lamivudine-$^{13}C,^{15}N_2$
Metformin	n.a.
Guanyl urea	Guanyl urea-$^{15}N_4$
N-Acetyl mesalazine	n.a.
Oxipurinol	Oxipurinol-$^{13}C,^{15}N_2$
Paracetamol	Paracetamol-d_4
Ranitidine	n.a.
Desmethyl ranitidine	n.a.
Ranitidine *N*-Oxide	n.a.
Ranitidine *S*-Oxide	n.a.

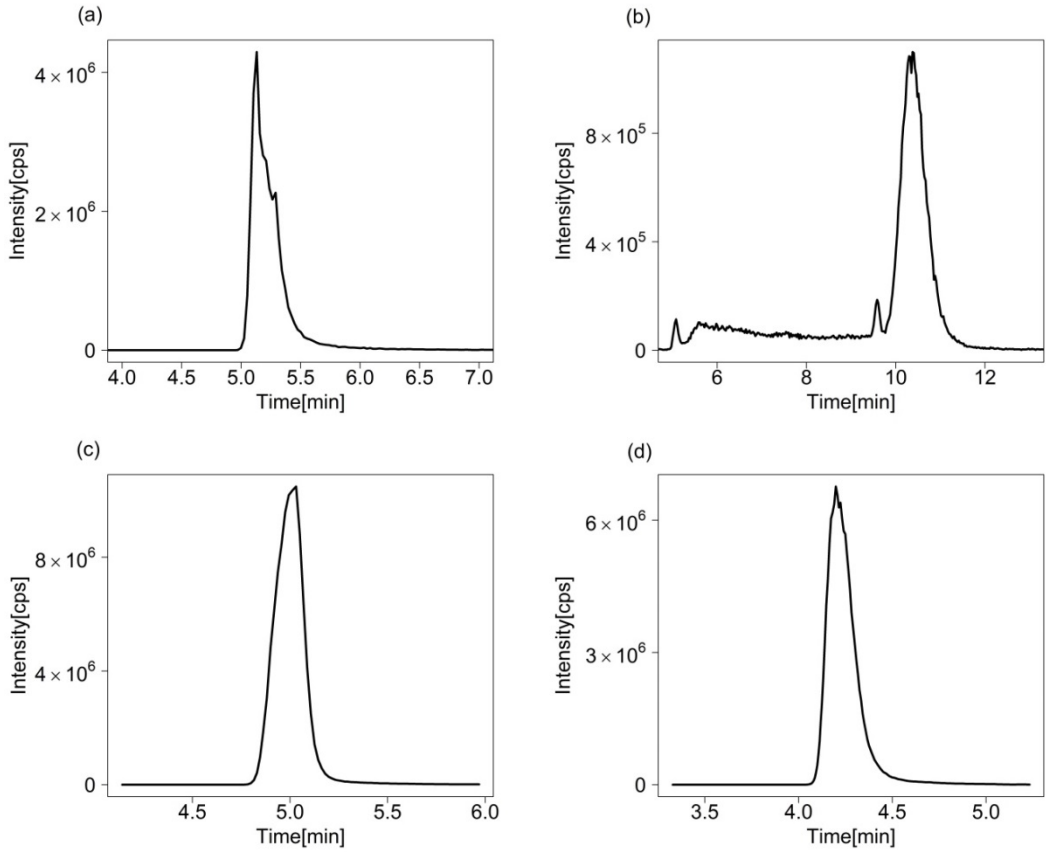


Figure A 1: Comparison of the peak form of 4-acetamidoantipyrine and 4-methylaminoantipyrine with and without ammonium formiate in B. (Source: Own representation, Federal Institute of Hydrology) (a) 4-acetamidoantipyrine without ammonium formiate in eluent B. (b) 4-methylaminoantipyrine without ammonium formiate in eluent B. (a) 4-acetamidoantipyrine without ammonium formiate in eluent B. (b) 4-methylaminoantipyrine without ammonium formiate in eluent B. (c) 4-acetamidoantipyrine with ammonium formiate in eluent B. (d) 4-methylaminoantipyrine with ammonium formiate in eluent B. Conditions: column: HILIC Nucleodur (250 x 3 mm, 3 µm), eluent A (pH 3.3): 10 mM ammonium formiate, 0.1 % formic acid, eluent B: acetonitrile/Milli Q, 90/10, v/v, 7.5 mM ammonium formiate, 0.1 % formic acid, respectively acetonitrile/Milli Q, 90/10, v/v, 0.1 % formic acid, flow rate: 0.5 mL/min, gradient: 100 % B for 3 min, 100 - 75 % B in 14 min, 75 % B for 5 min and 100% B for 11 min. Detection via HILIC-ESI-MS/MS.

Table A 6: Retention time reproducibility.

Analytes	Retention time RSD [%]	
	Intra-day [%] (n=6)	Inter-day [%] (n=4)
4-Acetamidoantipyrine	0.00	0.71
4-Formylaminoantipyrine	0.09	0.55
4-Methylaminoantipyrine	0.14	0.65
9-Acridine carboxylic acid	0.05	0.47
Abacavir	0.10	0.63
Abacavir carboxylate	0.06	0.80
Acesulfame	0.12	0.42
Acyclovir	0.05	0.27
Bisoprolol	0.19	0.99
Clindamycin	0.05	0.20
Clindamycin sulfoxide	0.08	0.37
Diatrizoate	0.10	0.23
Emtricitabine	0.09	0.47
Emtricitabine carboxylate	0.09	0.30
Emtricitabine *S*-oxide	0.05	0.51
Gabapentin	0.04	0.54
Gabapentin lactam	0.13	0.52
Lamivudine	0.00	0.55
Metformin	0.09	0.52
Guanyl urea	0.09	0.37
N-Acetyl mesalazine	0.23	0.29
Oxipurinol	0.00	0.25
Paracetamol	0.12	0.45
Ranitidine	0.10	0.60
Desmethyl ranitidine	0.08	0.52
Ranitidine *N*-oxide	0.06	0.74
Ranitidine *S*-oxide	0.00	0.67

Table A 7: Recoveries of the analytes with the different investigated sample preparation procedures

Analytes	Recoveries (%)																
	Oasis MCX pH 2	Oasis MCX pH 3	Oasis MCX pH 5	Oasis HLB pH2	Oasis HLB pH3	Oasis HLB pH 5	Isolute ENV + pH	Isolute ENV + pH	Oasis WCX pH	Oasis WCX pH 7	Strata XCW pH	Strata XCW pH 7	HR-X pH 2	HR-X pH 3	HR-X pH 5	HR-X pH 8	Freeze-drying
4-Acetamidoantipyrine	70	110	100	87	97	101	62	69	83	81	75	66	73	89	115	107	107
4-Formylaminoantipyrine	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	95
4-Methylaminoantipyrine	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	94
9-Acridine carboxylic acid	90	96	114	37	90	87	4	69	84	80	90	81	88	83	99	93	120
Abacavir	112	125	126	86	101	102	0	79	87	92	83	91	68	84	108	107	95
Abacavir carboxylate	61	75	71	14	16	16	0	67	79	81	80	80	97	99	109	101	86
Acesulfame	2	2	0	2	9	2	7	1	0	0	0	0	29	75	126	19	102
Acyclovir	72	110	116	3	6	7	34	92	32	28	68	67	8	36	55	51	85
Bisoprolol	54	85	87	65	77	76	0	0	82	84	86	87	80	83	104	55	78
Clindamycin	58	71	67	77	85	84	0	2	0	6	39	42	84	83	110	104	98
Clindamycin sulfoxide	81	96	92	88	100	91	0	12	143	139	109	111	89	92	127	132	93
Diatrizoate	15	0	0	56	39	13	74	8	27	0	0	0	77	70	94	20	95
Emtricitabine	45	52	58	5	20	16	11	108	1	53	83	83	32	56	72	66	115
Emtricitabine carboxylate	125	68	28	17	50	3	22	6	2	3	10	13	65	91	26	4	108
Emtricitabine *S*-oxide	42	75	34	3	6	4	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	11	31	47	44	116
Gabapentin	83	76	71	3	5	3	0	62	85	75	83	81	20	18	34	35	115
Gabapentin lactam	72	87	79	84	88	93	92	88	54	38	63	44	75	98	123	116	109
Lamivudine	86	82	96	0	0	17	0	89	0	34	72	72	5	9	57	55	29
Metformin	1	0	0	1	0	1	0	0	45	55	51	53	0	0	1	7	112
Guanyl urea	99	92	82	3	3	3	0	0	75	79	80	70	n.a.	n.a.	n.a.	n.a.	73
N-Acetyl Mesalazine	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	90
Oxipurinol	0	1	1	0	0	0	43	60	9	9	5	5	0	0	2	0	100
Paracetamol	15	65	70	16	61	64	59	54	66	58	71	68	40	72	85	76	86
Ranitidine	49	38	50	21	32	58	0	0	0	0	2	1	26	56	72	65	82
Desmethyl-Ranitidine	41	32	38	7	16	35	0	0	0	0	4	5	15	27	47	34	85
Ranitidine *N*-oxide	48	36	55	20	42	56	0	0	0	0	4	5	26	41	68	74	50
Ranitidine *S*-oxide	48	28	35	1	2	4	1	1	1	1	1	1	7	5	42	44	109

Table A 8: Calibration information

Analytes	R^2	Range [ng/L]
4-Acetamidoantipyrine	0.99856	0.5-10000
4-Formylaminoantipyrine	0.9982	5-10000
4-Methylaminoantipyrine	0.99898	1-10000
9-Acridine carboxylic acid	0.99894	10-20000
Abacavir	0.9993	5-20000
Abacavir carboxylate	0.99634	20-10000
Acesulfame	0.99788	5-200000
Acyclovir	0.996	10-20000
Bisoprolol	0.99882	2-20000
Clindamycin	0.99828	0.5-10000
Clindamycin sulfoxide	0.99828	5-10000
Diatrizoate	0.99706	5-200000
Emtricitabine	0.99742	10-10000
Emtricitabine carboxylate	0.99912	10-10000
Emtricitabine *S*-oxide	0.9971	200-20000
Gabapentin	0.99556	200-100000
Gabapentin lactam	0.9986	5-20000
Lamivudine	0.99898	5-10000
Metformin	0.99906	50-10000
Guanyl urea (low calibration)	0.99106	100-10000
Guanyl urea (high calibration)	0.99672	2000-100000
N-Acetyl mesalazine	0.99922	50-20000
Oxipurinol	0.99574	200-200000
Paracetamol	0.99752	20-10000
Ranitidine	0.99798	0.5-20000
Desmethyl ranitidine	0.99876	1-10000
Ranitidine *N*-Oxide	0.99722	20-10000
Ranitidine *S*-Oxide	0.99908	10-20000

Table A 9: Multiple reaction monitoring parameters of chloride, nitrate and the sodium adduct of emtricitabine

Name	MRM	DP	CE	CXP	Polarity
Chloride 35	35/35	-65	-5	-29	Negative
Chloride 37	37/37	-75	-6	-3	Negative
Nitrate	62/62	-300	-6	-1	Negative
Emtricitabine sodium adduct	270/152	90	21	19	Positive

Table A 10: Influence of slight modification of the diluent on the analytes. MQ: Milli-Q, ACN: acetonitrile. Slight: Modification of peak height and width, medium: apparition of tailing in at least one condition, important: apparition of peak splitting

Analytes	RT (min)	Influence	Amelioration of peak form with increasing	Width 5 % [min]			Tailing factor		
				ACN/MQ (87.5/12.5)	ACN/MQ (90/10)	ACN/MQ (92.5/7.5)	ACN/MQ (87.5/12.5)	ACN/MQ (90/10)	ACN/MQ (92.5/7.5)
4-Acetamidoantipyrine	4.69	Important	ACN	0.62	0.4	0.36	2.2	1.1	1.1
4-Formylaminoantipyrine	4.26	Important	ACN	0.61	0.37	0.27	2.3	1.1	1.1
4-Methylaminoantipyrine	3.92	Important	MQ	0.12	0.33	0.47	1.2	1.3	0.7
9-Acridine carboxylic acid	10.65	Slight	ACN	0.41	0.38	0.37	1.1	1.1	1.0
Abacavir	5.43	Medium	ACN	0.52	0.38	0.25	1.6	1.2	1.1
Abacavir carboxylate	6.91	Not affected	-	0.36	0.37	0.36	1.3	1.3	1.3
Acesulfame	3.37	Not affected	-	0.30	0.31	0.32	1.1	1.1	1.1
Acyclovir	10.81	Slight	ACN	0.34	0.31	0.30	1.0	1.0	1.0
Bisoprolol	8.96	Medium	ACN	0.44	0.37	0.35	0.9	1.1	1.0
Clindamycin	12.50	Medium	ACN	0.27	0.24	0.22	1.4	1.2	1.1
Clindamycin sulfoxide	14.61	Not affected	-	0.26	0.26	0.26	0.9	0.9	0.9
Diatrizoate	14.28	Not affected	-	0.30	0.30	0.29	1.0	1.0	1.0
Emtricitabine	4.68	Important		0.59	0.35	0.32	2.2	1.2	1.1
Emtricitabine carboxylate	13.97	Not affected	-	0.33	0.32	0.32	1.1	1.1	1.1
Emtricitabine S-oxide	7.40	Slight	ACN	0.48	0.42	0.38	1.1	1.1	1.1
Gabapentin	13.05	Not affected	-	0.31	0.30	0.30	1.2	1.3	1.3
Gabapentin lactam	3.18	Slight	MQ	0.29	0.31	0.34	1.3	1.2	1.1
Lamivudine	6.95	Slight	ACN	0.49	0.38	0.32	1.0	1.1	1.1
Metformin	13.34	Not affected	-	0.31	0.31	0.32	1.1	1.1	1.2
Guanyl urea	14.20	Not affected	-	0.24	0.24	0.24	1.0	1.0	1.0
N-Acetyl mesalazine	6.77	Slight	ACN	1.06	1.01	0.97	1.6	2.0	2.3
Oxipurinol	5.20	Slight	ACN	0.49	0.39	0.34	1.0	1.0	1.0
Paracetamol	3.42	Slight	MQ	0.32	0.34	0.39	1.1	1.1	1.1
Ranitidine	12.75	Not affected	-	0.24	0.24	0.24	1.1	1.1	1.1
Desmethyl ranitidine	13.23	Not affected	-	0.27	0.27	0.27	1.1	1.1	1.1
Ranitidine N-oxide	13.75	Not affected	-	0.27	0.27	0.27	1.1	1.1	1.1
Ranitidine S-oxide	16.63	Not affected	-	0.27	0.27	0.27	1.2	1.2	1.2

Table A 11a: Results from the analysis of environmental samples. Concentration in µg/L.

Analyte	WWTP 1 (28/11/2016)	WWTP 1 (6-7/12/2016)	WWTP 1 (11	WWTP 1 (15	WWTP 2 (7/11/16)	WWTP 2 (8/11/16)	WWTP 2 (9/11/16)	WWTP 2 (10/11/16)	Saar (8/12/2016)	Saar (9/12/2016)	Saar (12/12/2016)	Saar (13/12/2016)	Saar (14/12/2016)
4-Acetamido-antipyrine	5.5 ± 0.1	1.8	1.4	1.7	0.29	0.51	0.41	0.52	0.26 ± 0.02	0.27 ± 0.02	0.29 ± 0.01	0.27 ± 0.02	0.28 ± 0.01
4-Formylamino-antipyrine	11 ± 0.1	7.6	9.0	9.9	10	8.8	9.1	8.2	0.36 ± 0.02	0.35 ± 0.02	0.368 ± 0.008	0.36 ± 0.04	0.36 ± 0.01
4-Methylamino-antipyrine	0.014 ± 0.001	0.018	< 0.02	0.015	0.014	0.055	0.04	0.017	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
9-Acridine carboxylic acid	0.279 ± 0.001	0.17	0.17	0.15	0.26	0.098	0.10	0.18	0.028 ± 0.001	0.031 ± 0.001	0.032 ± 0.001	0.032 ± 0.001	0.032 ± 0.001
Abacavir	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Abacavir carboxylate	0.17 ± 0.01	0.17	0.14	0.15	0.03	< 0.02	< 0.02	0.028	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
Acesulfame	3.4 ± 0.1	1.7	0.99	1.7	1.1	1.3	0.93	1.8	0.86 ± 0.06	0.85 ± 0.08	1.37 ± 0.09	1.01 ± 0.09	0.96 ± 0.05
Acyclovir	0.18 ± 0.01	0.11	0.047	0.069	0.072	0.14	0.068	0.25	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Bisoprolol	0.37 ± 0.01	0.38	0.38	0.41	0.2	0.24	0.19	0.23	0.031 ± 0.002	0.031 ± 0.001	0.035 ± 0.001	0.035 ± 0.001	0.036 ± 0.002
Clindamycin	0.12 ± 0.01	0.11	0.12	0.13	0.071	0.046	0.049	0.049	0.095 ± 0.01	0.065 ± 0.004	0.068 ± 0.001	0.176 ± 0.002	0.106 ± 0.002
Clindamycin sulfoxide	0.39 ± 0.04	0.29	0.25	0.22	0.31	0.20	0.25	0.34	0.049 ± 0.002	0.0538 ± 0.0005	0.058 ± 0.002	0.059 ± 0.005	0.0583 ± 0.0003
Diatrizoate	13.3 ± 0.2	12	14	19	0.093	0.078	0.061	< 0.05	1.04 ± 0.07	0.89 ± 0.06	1.01 ± 0.08	1.62 ± 0.06	1.49 ± 0.05
Emtricitabine	0.063 ± 0.001	0.13	0.096	0.12	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Emtricitabine carboxylate	1.0 ± 0.1	0.37	0.32	0.30	0.25	0.12	0.14	0.17	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Emtricitabine *S*-oxide	0.27 ± 0.04	0.38	0.27	0.30	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2
Gabapentin	7.3 ± 0.1	3.8	2.9	2.8	3.6	3.7	3.2	4.1	1.1 ± 0.04	1.16 ± 0.02	1.26 ± 0.04	1.25 ± 0.07	1.26 ± 0.07
Gabapentin lactam	0.68 ± 0.02	8.7	11	12	1.4	1.3	1.2	0.86	0.21 ± 0.02	0.22 ± 0.01	0.25 ± 0.01	0.25 ± 0.01	0.26 ± 0.004
Lamivudine	0.058 ± 0.00	0.041	0.031	0.04	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02

	1												
Metformin	4.2 ± 0.01	1.4	0.71	1.1	0.94	0.9	0.89	1.8	0.97 ± 0.03	1.02 ± 0.01	1.1 ± 0.01	1.04 ± 0.07	1.07 ± 0.02
Guanyl urea	76 ± 2	4.3	3.8	3.6	110	110	110	100	2.6 ± 0.1	2.7 ± 0.04	3.07 ± 0.03	3.3 ± 0.5	3.38 ± 0.07
N-acetyl mesalazine	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Oxipurinol	17 ± 1	2.1	2.5	2.4	28	26	30	27	1.4 ± 0.1	1.5 ± 0.3	1.7 ± 0.2	1.8 ± 0.2	2.0 ± 0.2
Paracetamol	< 0.25	< 0.25	< 0.25	< 0.25	< 0.25	< 0.25	< 0.25	< 0.25	< 0.25	< 0.25	< 0.25	< 0.25	< 0.25
Ranitidine	0.211 ± 0.001	0.30	0.21	0.23	0.13	0.11	0.11	0.13	0.0013 ± 0.0002	0.0011 ± 0.0003	0.0012 ± 0.0002	0.0016 ± 0.0002	0.0016 ± 0.0003
Desmethyl ranitidine	0.0089 ± 0.0003	0.011	0.0092	0.0092	0.0065	0.0063	0.0053	1.1	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Ranitidine *N*-oxide	0.037 ± 0.001	0.0075	0.0053	0.006	0.022	0.019	0.024	0.026	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Ranitidine *S*-oxide	0.038 ± 0.001	0.038	0.036	0.037	0.024	0.020	0.021	0.020	< 0.005	< 0.005	0.0086 ± 0.0002	0.0085 ± 0.0001	0.0085 ± 0.0001

Table A11b Results from the analysis of environmental samples. Concentration in µg/L.

Analyte	Rhine (km 592) 26/03-2/4/17	Rhine (km 590) 26/03-2/4/17	Rhine (km 590) December 2016	Rhine (km 482) 25/03/17-1/4/17	Horloff 1 (26/07/16. N 50.520°; E 9.043)	Horloff 2 (26/07/16. N 50.514° . E 8.950°)	Horloff 3 (26/07/16.. N 50.411° . E 8.901°)	Horloff 4 (26/07/16.. N 50.399°.E 8.899°)	Usa 1 (26/07/16.. N 50.317° . 8.524°)	Usa 2 (26/07/16.. N50.380°.E 8.713)	Usa 3 (26/07/16.. N 50.359°.E 8.744°)	Usa 4 (26/07/16.. 50.336°.E 8.771°)
4-Acetamidoantipyrine	0.087 ± 0.005	0.14 ± 0.005	0.171 ± 0.002	0.12 ± 0.006	< 0.001	0.17	0.16	0.13	0.011	0.9	0.85	0.82
4-Formylaminoantipyrine	0.081 ± 0.005	0.132 ± 0.01	0.214 ± 0.002	0.1 ± 0.004	< 0.002	0.25	0.22	0.12	< 0.002	1.0	0.99	4.0
4-Methylaminoantipyrine	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
9-Acridine carboxylic acid	0.0028 ± 0.0001	0.004 ± 0.0004	0.019 ± 0.001	0.0035 ± 0.0001	< 0.001	< 0.001	0.32	0.059	< 0.001	0.087	0.09	0.12
Abacavir	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Abacavir carboxylate	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Acesulfame	0.55 ± 0.08	0.58 ± 0.03	0.45 ± 0.01	0.50 ± 0.03	0.045	0.54	0.54	0.31	0.061	1.2	1.1	0.54
Acyclovir	0.0083 ± 0.0002	0.0073 ± 0.0003	0.0031 ± 0.0004	0.0076 ± 0.0006	< 0.002	< 0.002	< 0.002	< 0.002	< 0.002	< 0.002	< 0.002	0.07
Bisoprolol	0.0086 ± 0.0001	0.0084 ± 0.0001	0.0089 ± 0.0001	0.006 ± 0.0005	< 0.001	0.0033	0.02	0.011	< 0.0005	0.064	0.037	0.20
Clindamycin	0.014 ± 0.001	0.005 ± 0.0004	0.0117 ± 0.0003	0.003 ± 0.0001	< 0.0005	0.0074	0.018	0.017	< 0.0005	0.034	0.026	0.10
Clindamycin sulfoxide	0.009 ± 0.001	0.008 ± 0.002	0.0148 ± 0.001	0.0057 ± 0.0008	< 0.001	0.013	0.056	0.042	< 0.001	0.088	0.062	0.12
Diatrizoate	0.14 ± 0.04	0.15 ± 0.02	0.248 ± 0.005	0.12 ± 0.01	< 0.01	0.12	0.89	0.49	< 0.01	1.0	0.89	1.8
Emtricitabine	< 0.001	0.0005 ± 0.0001	0.0005 ± 0.0001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.0056	< 0.001	0.045
Emtricitabine carboxylate	< 0.01	0.021 ± 0.005	0.039 ± 0.004	0.017 ± 0.006	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.083	0.074	0.11
Emtricitabine S-oxide	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Gabapentin	0.3 ± 0.03	0.29 ± 0.04	0.373 ± 0.008	0.21 ± 0.04	< 0.05	0.26	0.88	0.43	< 0.05	2.1	2.1	3.3
Gabapentin lactam	0.034 ± 0.003	0.034 ± 0.001	0.057 ± 0.001	0.027 ± 0.002	< 0.01	0.26	1.3	0.81	< 0.01	0.40	0.42	0.57
Lamivudine	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Metformin	0.8 ± 0.1	0.42 ± 0.04	0.55 ± 0.01	0.37 ± 0.05	< 0.005	0.31	0.65	0.41	0.12	0.86	2.1	0.69

Guanyl urea	1.1 ± 0.2	0.96 ± 0.09	1.00 ± 0.02	0.8 ± 0.2	0.98	0.53	0.36	< 0.02	< 0.02	2.6	1.6	3.1
N-Acetyl mesalazine	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Oxipurinol	0.26 ± 0.06	0.19 ± 0.09	0.78 ± 0.04	< 0.2	< 0.2	< 0.2	5.0	2.2	< 0.2	4.6	1.9	5.1
Paracetamol	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
Ranitidine	0.0022 ± 0.0001	0.0023 ± 0.0002	0.0027 ± 0.0001	0.0012 ± 0.0001	< 0.0005	0.00060	0.010	0.0042	< 0.0005	0.0044	0.0026	0.06
Desmethyl ranitidine	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Ranitidine *N*-Oxide	< 0.005	< 0.005	0.0040 ± 0.0001	0.0035 ± 0.0001	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Ranitidine *S*-Oxide	0.0024 ± 0.0001	0.0026 ± 0.0001	0.0025 ± 0.0001	0.0025 ± 0.0002	< 0.001	< 0.001	0.0076	0.0039	< 0.001	< 0.001	< 0.001	0.0087

Table A11c Results from the analysis of environmental samples. Concentration in µg/L.

Analyte	Groundwater 1	Groundwater 2	Groundwater 3	Groundwater 4	Groundwater 5	Groundwater 6	Groundwater 7	Groundwater 8	Groundwater 9	Groundwater 10	Groundwater 11	Groundwater 12	Groundwater 13	Groundwater 14	Groundwater well -Coblence-Arenberg
4-Acetamidoantipyrine	< 0.001	< 0.001	0.0072 ± 0.002	< 0.001	< 0.001	0.014	0.036	0.0017	0.038	0.063	0.040	0.0092	0.0033	0.012	< 0.001
4-Formylaminoantipyrine	0.035 ± 0.001	0.025	0.044	0.0033	< 0.001	0.09	0.23	0.044	0.19	0.25	0.16	0.064	0.014	0.21	< 0.001
4-Methylaminoantipyrine	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
9-Acridine carboxylic acid	0.0064 ± 0.0007	0.0031	0.045	< 0.001	< 0.001	0.14	0.39	0.14	0.32	0.031	0.41	0.16	0.028	0.045	< 0.001
Abacavir	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Abacavir carboxylate	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.011	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Acesulfame	0.35 ± 0.02	0.25	1.3	0.042	0.21	1.4	0.38	0.22	0.3	0.24	0.39	0.25	0.86	6.1	< 0.001
Acyclovir	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Bisoprolol	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.0026	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Clindamycin	< 0.0001	0.00022	< 0.0001	< 0.0001	< 0.0001	0.00082	0.00089	< 0.0001	0.0031	0.010	0.0024	0.0001	< 0.0001	< 0.0001	< 0.0001
Clindamycin sulfoxide	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.0011	< 0.001	0.0032	0.010	0.0031	0.0011	< 0.001	< 0.001	< 0.001
Diatrizoate	0.21 ± 0.03	0.18	1.2	0.061	0.08	0.05	0.01	0.11	< 0.01	0.16	< 0.01	0.054	0.21	< 0.01	< 0.01
Emtricitabine	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.0019	0.0035	< 0.001	< 0.001	< 0.001	0.0039	< 0.001	< 0.001	< 0.001	< 0.001
Emtricitabine carboxylate	0.0058	0.0052	< 0.005	< 0.005	< 0.005	0.26	0.37	0.087	0.23	0.13	0.31	0.086	0.29	0.30	< 0.005
Emtricitabine *S*-oxide	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.019	0.023	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Gabapentin	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	1.1	2.7	0.26	0.76	0.37	0.96	0.41	0.14	3.0	< 0.05
Gabapentin lactam	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.061	0.14	0.016	0.086	0.033	0.12	0.026	0.013	0.051	< 0.01
Lamivudine	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.0018	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.0017	< 0.001
Metformin	0.026 ± 0.002	0.064	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	0.14	0.0076	0.16	< 0.005	0.16	< 0.005	< 0.005	< 0.005
Guanyl urea	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	0.032	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
N-acetyl mesalazine	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Oxipurinol	1.1 ± 0.1	1.3	0.66	< 0.05	< 0.05	0.21	0.21	1.1	1.8	0.084	1.1	1.6	< 0.05	< 0.05	< 0.05
Paracetamol	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Ranitidine	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0043	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Desmethyl ranitidine	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Ranitidine *N*-oxide	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Ranitidine *S*-oxide	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Table A 12: Overview over middle polar analytes

Parent	Metabolite / transformation product				
Aliskiren					
Amisulpride	O-Desmethyl-Amisulpride				
Amoxicillin	Amoxicillin-2',5'-diketopiperazine	Amoxilloic acid			
Aripiprazol	Dehydroaripiprazol	Aripiprazol-N-Oxide			
Atenolol	Atenolol acid	OH-Atenolol			
Azithromycin	N-Desmethylazithromycin	Azithromycin-N-Oxide			
Bezafibrat	4-Chlorobenzoic acid	4-hydroxybenzoic acid	3-[(4-chlorobenzoyl) amino]propanoic acid		-
Bicalutamide					
Bosentan	Desmethylbosentan	Hydroxylbosentan			
Candesartan					
Carbamazepine	10, 11-Dihydro-10;11 - transdihydroxycarbamazepine	2-Hydroxycarbamazepine	3-Hydroxycarbamazepine	10-Hydroxycarbamazepine	Acridone
Cefuroxim	Descarbamoyl Cefuroxim				
Cetirizin	Cetirizin-N-oxide				
Citalopram	N-Desmethylcitalopram	Didesmethym-Citalopram	Citalopram-N-Oxide		
Clarithromycin	14-Hydroxy-(R)-clarithromycin	N-Desmethylclarithromycin			
Clopidogrel	Clopidogrel acid				
Diclofenac	4-hydroxy-diclofenac	Carboxy-diclofenac	Diclofenac Lactam		
Diphenhydramine	N-Desmethyl-Diphenhydramine	Diphenhydramine-N-Oxid			-
Dipyridamole					
Duloxetin	4-Hydroxy Duloxetin				

Ausgangssubstanz	Metaboliten/Transformationsprodukte				
Enalapril	Enalaprilat				
Erythromycin	Dehydro-Erythromycin				
Fexofenadine					
Flecainide	M-O-dealkylated Flecainide				
Fluconazole					
Fluoxetin	Norfluoxetin				
Furosemide					
Hydrochlorothiazid	Chlorothiazid	4-amino-6-chloro-1,3-benzenedisulfonamid			
Ibuprofen	2-Hydroxy-Ibuprofen	Carboxyibuprofen	4-acetylbenzoic acid		
Imatinib	N-Desmethyl imatinib				
Irbesartan					
Lamotrigine	oxo-lamotrigin	lamotrigine N2-glucoronide	N2-Methyl Lamotrigine		
Lapatinib					
Levetiracetam	Levetiracetam carboxylic Acid				
Lidocaine	Nor-Lidocaine				
Methylphenidat	Ritalinic acid				
Metoprolol	α-hydroxymetoprolol	O-desmethylmetoprolol	Metoprolol acid		
Naproxen	O-Desmethyl Naproxen				
Olmesartan					
Oxazepam					
Phenytoin					
Primidone					
Ausgangssubstanz	**Metaboliten/Transformationsprodukte**				
Quetiapin	7-Hydroxyquetiapin	Quetiapinesulfoxid	Norquetiapine		

Ramipril	Ramiprilat				
Roxithromycin	O-Desmethylroxithromycin	N-Desmethylroxithromycin			
Sertralin	Desmethylsertralin	N-hydroxysertralin	Sertralin ketone		
Sildenafil	N-Desmethyl sildenafil	N-dealkylated sildenafil			
Simvastatin	Hydroxymethyl-Simvastatin	Dehydro-Simvastatin			
Sitagliptin					
Ramipril	Ramiprilat				
Sotalol					
Sulfamethoxazol	N-Hydroxysulfamethoxazol	N4-Acetylsulfamethaxol			
Sulpiride					
Tadalafil	Desmethyltadalafil				
Telmisartan					
Thyroxin					
Tolylbiguanide					
Torasemide	Hydroxy-Torasemide				
Tramadol	O-Desmethyltramadol	Dehydro-Tramadol		N-Desmethyltramadol	Didesmethyltramadol (N,O und N,N)
Trimethoprim	3-Desmethyl-Trimethoprim	5-(2,4,5-Trimethoxy)-2,4-pyrimidinediamnine			
Valsartan	4-hydroxyvalsartan	Valsartan acid			
Venlafaxin	O-Desmethylvenlafaxine	N-Desmethylvenlafaxine	3-[(4-chlorobenzoyl) amino]propanoic acid	N,O-Didesmethylvenlafaxine	Venlafaxine-N-Oxide
Xipamide					

Table A 13: Overview of the two sensitiv mass transitions (MRM 1 und 2) and corresponding optimized mass spectrometric; MRM: Multiple Reaction Monitoring, DP: Declustering potential, CE: Collision Energy, CXP: Cell Exit Potential

Substanz	MRM 1 [m/z]	MRM 2 [m/z]	DP [V]	CE [eV] (MRM 1/ MRM 2)	CXP [V] (MRM 1/ MRM 2)	Polarität
10,11-dihydroxy-10,11-dihydrocarbamazepine	271/236	271/180	41	19/45	6/12	Positiv
10-Hydroxy-Carbamazepine	255.2/179.1	255.2/194.1	46	52/27	14/14	Positiv
14-(R)-Hydroxyclarithromycin	748.5/158	748.5/116	74	44/67	11/8	Positiv
2-Hydroxy-Carbamazepine	253.1/210,2	253.1/208	71	29/35	12/18	Positiv
3-Desmethyl-Trimethoprim	277.1/261.1	277.1/123.1	86	38/51	15/10	Positiv
3-Hydroxy-Carbamazepine	253.1/210.1	253.1/167	66	27/51	14/10	Positiv
4'-Hydroxy-Diclofenac	312/230	312/231	47	46/28	17/17	Positiv
4-Hydroxy-Duloxetin	314.1/154	314.1/204	45	10/9	12/17	Positiv
5-(3,4,5-Trimethoxybenzoyl)-2,4-pyrimidinediamine	305.1/137	305.1/244.1	80	35/35	7/6	Positiv
7-Hydroxy-Quetiapine	400.1/269	400.1/208	80	35/65	5/14	Positiv
Acetyl-Sulfamethaxol	296.1/134	296.1/198	81	35/25	12/14	Positiv
Acridone	196/167.1	196/139.1	96	43/71	30/22	Positiv
Aliskiren	552.4/436.3	552.4/534.4	65	28/28	12/12	Positiv
Amisulprid	370.2/242	370.2/196	106	39/59	14/12	Positiv
Aripiprazol	448.2/285	448.2/176	95	39/48	7/13	Positiv
Aripiprazol-N1-Oxid	464.1/243	464.1/218	100	45/33	5/12	Positiv
Aripiprazol-N4-Oxid	464/243	464/285	70	40/30	5/7	Positiv
Atenolol	267/145	267/190	61	37/27	12/16	Positiv
Atenololsäure	268.1/191.2	268.1/226.1	56	27/25	16/20	Positiv
Azithromycin	749.5/591.4	749.5/158.1	100	40/55	8/8	Positiv
Azithromycin-N-Oxid	765.5/546	765.5/607	90	45/34	16/18	Positiv

Substanz	MRM 1 [m/z]	MRM 2 [m/z]	DP [V]	CE [eV] (MRM 1/ MRM 2)	CXP [V] (MRM 1/ MRM 2)	Polarität
Candesartan	441.2/263.2	441.2/207.2	51	17/35	16/12	Positiv
Carbamazepin	237.1/194	237.1/179.1	71	27/49	16/12	Positiv
Carboxy-Diclofenac	282/229	282/264	28	37/14	16/14	Positiv
Cetirizine	389.1/201.1	389.1/166.1	55	30/60	10/10	Positiv
Cetirizin-N-Oxid	405/201	405/166	80	39/73	15/12	Positiv
Citalopram	325.2/109.1	325.2/262.1	85	37/27	10/10	Positiv
Citalopram-N-Oxid	341.2/109.1	341.2/262.1	60	35/27	8/6	Positiv
Clarithromycin	748.5/158.1	748.5/590.4	86	39/27	14/12	Positiv
Clopidogrel	322.1/212	322.1/184	31	23/31	14/12	Positiv
Clopidogrelsäure	308/198	308/152	66	23/33	12/10	Positiv
D,L,O-Desmethyl-Venlafaxine	264/246	264/58	56	19/39	18/11	Positiv
Dehydroaripiprazol	446.2/285	446.2/98	85	36/61	7/7	Positiv
Dehydro-Erithromycin	716.5/158	716.5/116	80	45/66	13/8	Positiv
Dehydro-Simvastatin	401.3/199	401.3/285	80	22/13	16/7	Positiv
Dehydrotramadol	246.2/121	246.2/115	50	42/82	9/8	Positiv
Desmethyl-Citalopram	311.1/109.1	311.1/262.1	45	32/26	10/10	Positiv
Desmethyl-Sertralin	292/159	292/275	51	37/13	10/14	Positiv
Diclofenac	296/215	296/250	46	27/19	15/15	Positiv
Diclofenac Lactam	278/214	278/215	60	39/30	16/13	Positiv
Didesmethyl-Citalopram	297.1/109	297.1/116	60	30/30	6/6	Positiv
Diphenhydramine	256.2/167	256.2/152	20	20/50	5/8	Positiv
Diphenhydramine N-oxide	272.2/167	272.2/88	35	25/17	10/5	Positiv
Duloxetin	298.2/154	298.2/188	40	10/9	12/15	Positiv

Substanz	MRM 1 [m/z]	MRM 2 [m/z]	DP [V]	CE [eV] (MRM 1/ MRM 2)	CXP [V] (MRM 1/ MRM 2)	Polarität
Enalapril	377/234	377/303	71	27/26	12/16	Positiv
Erythromycin	734.5/158.1	734.5/576.4	86	43/29	12/22	Positiv
Fexofenadin	502.3/466.3	502.3/171.1	80	38/57	5/5	Positiv
Flecainide	415.2/398.1	415.2/301	80	35/50	10/10	Positiv
Flecainide-meta-O-dealkylated	333.1/316.1	333.1/219.1	60	28/40	8/8	Positiv
Fluconazol	307.1/238,1	307.1/220.1	70	20/25	20/15	Positiv
Fluoxetin	310/44	310/148	45	30/14	10/10	Positiv
Hydroxyatenolol	283.1/116	283.1/74	65	25/40	8/8	Positiv
Hydroxy-Metoprolol	284.2/116	284.2/74	70	28/35	5/5	Positiv
Hydroxy-Simvastatin	435.3/319	435.3/197	80	13/25	9/16	Positiv
Hydroxy-Torasemid	365.1/280,1	365.1/306,1	50	25/20	8/8	Positiv
Imatinib	494.3/394	494.3/217	90	39/37	11/18	Positiv
Lamotrigine	256/211	256/157	80	38/45	10/10	Positiv
Lapatinib	581.1/365	581.1/350	130	54/54	9/9	Positiv
Levetiracetam	171.1/126.1	171.1/154.1	76	19/11	8/10	Positiv
Levetiracetam acid	172.1/126	172.1/69.2	96	19/33	8/8	Positiv
Lidocaine	235.2/86.1	235.2/58.1	80	23/53	14/2	Positiv
Metoprolol	268/116	268/74	75	27/35	10/11	Positiv
N,O-Desmethyl-Tramadol	236.1/44	-	44	20/32	8/10	Positiv
N,O-Desmethyl-Venlafaxine	250.2/44.2	250.2/132.9	36	32/31	10/10	Positiv
N-Desmethyl-Azithromycin	735.5/434	735.5/559	90	55/48	12/8	Positiv
N-Desmethyl-Clarithromycin	734.4/144	734.4/365	80	38/35	11/9	Positiv
N-Desmethyl-Diphenhydramin	242/167	242/152	30	20/50	10/10	Positiv

Substanz	MRM 1 [m/z]	MRM 2 [m/z]	DP [V]	CE [eV] (MRM 1/ MRM 2)	CXP [V] (MRM 1/ MRM 2)	Polarität
N-Desmethyl-Imatinib	480.3/394	480.3/203	90	40/37	11/16	Positiv
N-Desmethyl-Roxithromycin	823.5/144	823.5/666	85	40/35	10/10	Positiv
N-Desmethyl-Sildenafil	461/283	461/311	115	53/43	7/8	Positiv
N-Desmethyl-Tramadol	250.1/44	-	45	20/55	11/11	Positiv
N-Desmethyl-Venlafaxine	264.1/44	264.1/121.1	36	55/37	11/10	Positiv
Norfluoxetin	296/259	296/134	55	24/11	10/10	Positiv
Nor-Lidocaine	207.1/58	207.1/122.1	35	30/20	8/8	Positiv
Norquetipaine	296/210	296/253	80	42/33	11/6	Positiv
O-Desmethyl Amisulprid	356.2/112.1	356.2/129.1	166	37/31	8/22	Positiv
O-Desmethyl-Metoprolol	254.2/177	254.2/116	70	25/25	10/8	Positiv
O-Desmethyl-Tramadol	250.1/58	-	45	20/45	11/8	Positiv
O-Desmethyl-Venlafaxine	264.1/58	264.1/107	56	45/45	8/8	Positiv
Oxazepam	287.1/241	287.1/104	61	47/81	8/6	Positiv
Pregabalin	160.1/55	160.1/97	41	35/21	10/5	Positiv
Primidon	219/162	219/91	40	16/39	13/13	Positiv
Quetiapin	384.2/253.2	384.2/221.3	80	30/60	11/15	Positiv
Quetipinesulfoxid	400/221	400/269	90	50/29	5/6	Positiv
Ramipril	417.2/234.1	417.2/343.2	70	28/30	10/10	Positiv
Ramiprilat	389.2/206.4	389.2/156.4	60	30/28	13/10	Positiv
Ritalinic acid	220,1/84,1	220.1/84.1	60	27/27	10/10	Positiv
Roxithromycin	837.5/158	837.5/679	106	47/29	11/26	Positiv
Sertralin	306.3/159	306.3/275.1	63	37/20	13/7	Positiv
Sertralinketon	291/145	291/117	80	29/40	10/8	Positiv

Substanz	MRM 1 [m/z]	MRM 2 [m/z]	DP [V]	CE [eV] (MRM 1/ MRM 2)	CXP [V] (MRM 1/ MRM 2)	Polarität
Sildenafil	475.3/100	475.3/283	100	43/58	6/7	Positiv
Simvastatin	419.3/285	419.3/199	80	16/19	8/17	Positiv
Sitagliptin	408.1/235.1	408.1/174	51	29/33	38/24	Positiv
Sotalol	273/134	273/213	46	37/26	10/10	Positiv
Sulfamethaxol	254.1/156	254.1/188	66	23/21	12/14	Positiv
Sulpirid	342.2/112,1	342.2/214	60	35/45	8/10	Positiv
Telmisartan	515.2/497.2	515.2/276.1	181	45/61	22/26	Positiv
Thyroxin	777.7/731.7	777.7/605	110	35/57	20/10	Positiv
Torasemid	349.1/264.1	349.1/290.1	60	25/20	8/8	Positiv
Tramadol	264.2/58	280.2/262.2	46	45/18	4/12	Positiv
Tramadol-N-oxid	280.2/262.2	280.2/135	50	18/35	12/12	Positiv
Trimethoprim	291.1/230.1	291.1/261.1	86	33/35	11/10	Positiv
Valsartan	436,2/235,1	436,2/207.1	111	27/35	12/16	Positiv
Valsartanic acid	267.1/151.1	278.2/58	80	57/43	10/8	Positiv
Venlafaxin	278.2/58	278.2/121.1	36	43/28	8/8	Positiv
Venlafaxin-N-oxid	294.2/178.1	294.2/121.1	50	25/35	12/8	Positiv
2-Hydroxy-Ibuprofen	221/177	303/177	-30	-11/-20	-5/-8	Negativ
3-[(4-chlorobenzoyl) amino]propanoic acid	226/154.1	228/156.1	-45	-20/-20	-10/-10	Negativ
4-amino-6-chloro-1,3-benzenedisulfonamide	284/78	286/78	-70	-50/-50	-4/-4	Negativ
4-Chlorobenzoic acid	155/111	157/113	-35	-18/-16	-5/-7	Negativ
4-hydroxybenzoic acid	137/93	137/65	-40	-18/-45	-5/-8	Negativ
4-Hydroxy-Valsartan	450.5/350	450.5/179	-75	-26/-40	-3/-8	Negativ
Amoxicillin	364/223	364/206	-55	-15/-22	-11/-13	Negativ

Substanz	MRM 1 [m/z]	MRM 2 [m/z]	DP [V]	CE [eV] (MRM 1/ MRM 2)	CXP [V] (MRM 1/ MRM 2)	Polarität
Amoxicillin-2,5-diketopiperazin	364/330	364/286	-50	-15/-18	-3/-7	Negativ
Amoxicilloic acid	382/338	382/260	-35	-13/-20	-9/-19	Negativ
Bezafibrate	360.1/274.1	360.1/154	-65	-22/-36	-17/-9	Negativ
Bicalutamide	429.1/185	429.1/255	-55	-50/-22	-9/-13	Negativ
Bosentan	550.2/197	550.2/308	-90	-44/-47	-4/-6	Negativ
Carboxy-Ibuprofen	235/73	191/73	-40	-20/-20	-10/-10	Negativ
Cerfuroxim	423/207	423/318	-40	-16/-13	-10/-7	Negativ
Chlorothiazide	294/214	294/179	-80	-40/-62	-4/-10	Negativ
Descarbamoylcefuroxim	380/207	380/336	-35	-18/-11	-15/-9	Negativ
Desmethylbosentan	536.5/212	536.5/197	-90	-40/-56	-4/-9	Negativ
Desmethylen-Tadalafil	376.3/266	376.3/235	-90	-21/-44	-6/-4	Negativ
Furosemide	329/285	329/205	-90	-20/-30	-13/-9	Negativ
Hydrochlorothiazide	296/268.9	296/205	-120	-26/-32	-13/-11	Negativ
Hydroxylbosentan	566.2/213	566.2/149	-100	-45/-57	-4/-6	Negativ
Ibuprofen	205.1/161	427.2/121	-30	-10/-6	-11/-9	Negativ
Irbesartan	427.2/193.1	229.1/170	-70	-35/-80	-6/-4	Negativ
Naproxen	229.1/185	215/171	-50	-11/-22	-13/-11	Negativ
O-Desmethyl-Naproxen	215/169	445.2/167.1	-35	-40/-11	-8/-8	Negativ
Olmesartan	445.2/149.1	255/164	-50	-50/-35	-6/-6	Negativ
Oxo-Lamotrigine	255/219	251/208	-57	-16/-23	-4/-11	Negativ
Phenytoin	251/102	388.1/232	-45	-28/-25	-5/-5	Negativ
Tadalafil	388,1/262	353.1/127	-80	-26/-53	-6/-4	Negativ
Xipamide	353.1/274.1	427.2/121	-60	-36/-45	-8/-8	Negativ

Table A 14: LC-MS/MS detection method and further information of steroid hormones investigated. (TRC= Toronto Research Chemicals, Canada Ontario; SC= Santa Cruz Biotechnology, USA Texas; SA= Sigma-Aldrich, Germany Munich)

Abbre-viation	Substance	Sup-plier	CAS-No.	Chemical formula	Applica-tion quantity in GER [kg in 2014]	log D (pH 7)[6]	Internal standard used for correction	Reten-tion time [min]	Adduct	Pre-cursor [Da]	Fragment mass [Da]	Collision energy [V]	Declus-tering poten-tial [V]
Progestogens (PG)													
CLM	**Chlormadinone**	TRC	1961-77-9	$C_{21}H_{27}ClO_3$	-	3.28	d4-E1	21.2	$[M-H]^-$	361	333/287	-27/-30	-40
CLMac	**Chlormadinone acetate**	TRC	302-22-7	$C_{23}H_{29}ClO_4$	99	3.72	d5-CLOprop	22.3	$[M+H]^+$	405	309/267	22/32	90
CYP	**Cyproterone**	SC	2098-66-0	$C_{22}H_{27}ClO_3$	-	3.20	d5-CLOprop	20.9	$[M+H]^+$	375	321//293	28/32	110
CYPac	**Cyproterone acetate**	SA	427-51-0	$C_{24}H_{29}ClO_4$	99	3.64	d3-CYPac	22.0	$[M+H]^+$	417	357/321	23/27	100
DIE	**Dienogest**	SA	65928-58-7	$C_{20}H_{25}NO_2$	278	2.31	d8-DIE	17.5	$[M+H]^+$	312	161/135	38/40	160
DIE-m1	**6ß-Hydroxy dienogest**	SC	-	$C_{20}H_{25}NO_3$	-	1.08	d8-DIE	12.7	$[M+H]^+$	328	107/251	33/33	60
DPN	**Drospirenone**	SA	67392-87-4	$C_{24}H_{30}O_3$	61	3.37	13C3-DPN	20.6	$[M+H]^+$	367	97/197	30/30	90
ETG	**Etonogestrel**	SA	54048-10-1	$C_{22}H_{28}O_2$	0.4	3.60	d6-LNG	21.2	$[M+H]^+$	325	257/197	25/27	80
GES	**Gestodene**	SA	60282-87-3	$C_{21}H_{26}O_2$	-	3.46	13C3-DPN	20.3	$[M+H]^+$	311	109/201	32/26	100
HPG	**17α-Hydroxy progeste-rone**	SA	68-96-2	$C_{21}H_{30}O_3$	-	3.40	d6-LNG	20.6	$[M+H]^+$	331	109/97	34/28	80
LNG	**Levonorgestrel**	SA	797-63-7	$C_{21}H_{28}O_2$	17	3.66	d6-LNG	20.9	$[M+H]^+$	313	245/109	25/32	120
MPR	**Medroxy progesterone**	SA	520-85-4	$C_{22}H_{32}O_3$	-	3.69	d6-LNG	21.4	$[M+H]^+$	345	123/97	33/50	100
MPRac	**Medroxy progesterone acetate**	SA	71-58-9	$C_{24}H_{34}O_4$	570	4.13	d3-CYPac	22.3	$[M+H]^+$	387	327/123	20/40	100
MPRac-m1	**6ß-Hydroxy medroxy progesterone acetate**	TRC	984-47-4	$C_{24}H_{34}O_5$	-	2.89	d4-E1	19.7	$[M-H]^-$	401	359/341	-25/-36	-75
MEG	**Megestrol**	TRC	3562-63-8	$C_{22}H_{30}O_3$	-	3.28	d4-E1	21.0	$[M-H]^-$	341	313/255	-26/-25	-90
MEGac	**Megestrol acetate**	TRC	595-33-5	$C_{24}H_{32}O_4$	-	3.72	d3-CYPac	22.1	$[M+H]^+$	385	224/267	40/26	80
NES	**Norethisterone**	SA	68-22-4	$C_{20}H_{26}O_2$	12	3.22	d6-NES	19.8	$[M+H]^+$	299	231/109	25/32	110
NESac	**Norethisterone acetate**	SA	51-98-9	$C_{22}H_{32}O_4$	9	3.66	d10-BMSdiprop	22.2	$[M+H]^+$	341	281/109	20/40	110
Glucocorticoids (GC)													

BEC	**Beclomethasone**	SA	4419-39-0	$C_{22}H_{29}ClO_5$	-	2.15	d3-FMS	17.2	$[M+HCOO]^-$	453	377 / 297	-20 / -34	-10
BECprop	**Beclomethasone 17-propionate**	TRC	5534-18-9	$C_{25}H_{33}ClO_6$	-	3.29	d5-CLOprop	20.4	$[M+H]^+$	465	355 / 337	16 / 20	40
BECdiprop	**Beclomethasone 17,21-dipropionate**	TRC	5534-09-8	$C_{28}H_{37}ClO_7$	158	4.43	d10-BECdiprop	22.4	$[M+H]^+$	521	411 / 319	15 / 25	70
BMS	**Betamethasone**	SA	378-44-9	$C_{22}H_{29}FO_5$	7	1.68	d5-DMS	16.4	$[M+HCOO]^-$ /$[M+H]^+$	437 /393	361 /373	-23 /17	-10 /70
BMSac	**Betamethasone 21-acetat**	SA	987-24-6	$C_{24}H_{31}FO_6$	7	2.12	d3-BMSac	19.7	$[M+H]^+$	435	415 / 397	12 / 15	40
BMSval	**Betamethasone 17-valerat**	SA	2152-44-5	$C_{27}H_{37}FO_6$	98	3.71	d5-CLOprop	21.3	$[M+H]^+$	477	355/337	18/20	60
BMSprop	**Betamethasone 17-propionat**	TRC	5534-13-4	$C_{25}H_{33}FO_6$	-	2.82	d5-BMSprop	19.9	$[M+H]^+$	449	429/355	11/16	70
BMSdiprop	**Betamethasone 17,21-dipropionate**	SA	5593-20-4	$C_{28}H_{37}FO_7$	116	3.96	d10-BMSdiprop	22.1	$[M+H]^+$	505	411/485	17/14	50
BDN	**Budesonide**	SA	51333-22-3	$C_{25}H_{34}O_6$	354	2.73	d8-BDN	19.9 (20.0)	$[M+H]^+$	431	323/147	20/35	30
BDN-m1	**6ß-Hydroxy budesonide**	SC	88411-77-2	$C_{25}H_{34}O_7$	-	1.50	13C3-TRIact	15.4	$[M+H]^+$	447	339/357	17/17	50
CIC	**Ciclesonide**	TRC	126544-47-6	$C_{32}H_{44}O_7$	1	5.32	d10-BECdiprop	24.8	$[M+H]^+$	541	323/305	25/30	80
CIC-m1	**Desisobutyryl ciclesonide**	TRC	161115-59-9	$C_{28}H_{38}O_6$	-	3.64	d5-CLOprop	21.8	$[M+H]^+$ /$[M+HCOO]^-$	471 /515	323 /357	25 /-20	80 /-40
CLO	**Clobetasol**	SA	25122-41-2	$C_{22}H_{28}ClFO_4$	-	3.04	d6-LNG	20.4	$[M+H]^+$	411	373/171	20/29	60
CLOprop	**Clobetasol 17-propionate**	SA	25122-46-7	$C_{25}H_{32}ClFO_5$	89	4.18	d5-CLOprop	21.8	$[M+H]^+$	467	373/355	16/20	50
HCOR	**Cortisol (Hydrocortisone)**	SA	50-23-7	$C_{21}H_{30}O_5$	605	1.28	d8-PNL	14.5	$[M+HCOO]^-$ /$[M+H]^+$	407 /363	331 /121	-23 /32	-20 /110
COR	**Cortisone**	SA	53-06-5	$C_{21}H_{28}O_5$	-	1.66	d8-PNL	14.8	$[M+HCOO]^-$	405	329/301	-15/-27	-10
DMS	**Dexamethasone**	SA	50-02-2	$C_{22}H_{29}FO_5$	277	1.68	d5-DMS	16.6	$[M+HCOO]^-$ /$[M+H]^+$	437 /393	361 /373	-23 /17	-10 /70
DMS-m1	**6ß-Hydroxy dexamethasone**	TRC	55879-87-3	$C_{22}H_{29}FO_6$	-	0.45	d5-DMS	10.8	$[M+HCOO]^-$	453	377/308	-24/-45	-40
DMSac	**Dexamethasone 21-acetate**	SA	1177-87-3	$C_{24}H_{31}FO_6$	3	2.12	d3-BMSac	20.0	$[M+H]^+$	435	415/397	12/15	40
DFCval	**Diflucortolone 21-valerate**	TRC	59198-70-8	$C_{27}H_{36}F_2O_5$	3	4.04	-	22.5	$[M-H]^-$	477	457/373	-14/-23	-40

FMS	**Flumethasone**	SA	2135-17-3	$C_{22}H_{28}F_2O_5$	-	1.34	d3-FMS	16.8	$[M+HCOO]^-$	455	379/305	-25/-50	-40
FMSpiv	**Flumethasone 21-pivalate**	SA	2002-29-1	$C_{27}H_{36}F_2O_6$	1	3.58	-	21.9	$[M-H]^-$	493	371/101	-23/-55	-90
FCNact	**Fluocinolone acetonide**	SA	67-73-2	$C_{24}H_{30}F_2O_6$	12	1.60	13C3-TRIact	18.2	$[M+H]^+$	453	413/433	17/13	80
FML	**Fluorometholone**	SA	426-13-1	$C_{22}H_{29}FO_4$	3	1.34	d4-E1	18.7	$[M-H]^-$	375	355/255	-12/-20	-50
FLUfur	**Fluticasone 17-furoate**	TRC	397864-44-7	$C_{27}H_{29}F_3O_6S$	2	4.13	d5-FLUprop	21.8	$[M+H]^+$	539	313/293	17/29	80
FLUprop	**Fluticasone 17-propionate**	SA	80474-14-2	$C_{25}H_{31}F_3O_5S$	80	3.72	d5-FLUprop	21.8	$[M+H]^+$	501	313/293	20/25	80
HAL	**Halcinonide**	TRC	3093-35-4	$C_{24}H_{32}ClFO_5$	-	3.30	-	21.7	$[M-H]^-$	453	433/309	-33/-44	-120
HLM	**Halometasone**	TRC	50629-82-8	$C_{22}H_{27}ClF_2O_5$	1	1.73	d4-E1	19.2	$[M-H]^-$	443	413/362	-12/-35	-20
MPNL	**Methylprednisolone**	SA	83-43-2	$C_{22}H_{30}O_5$	157	1.56	d3-FMS	15.9	$[M+HCOO]^-$	419	343/294	-23/-47	-10
MPNLacp	**Methylprednisolone 21-acetate 17-propionate**	TRC	86401-95-8	$C_{27}H_{36}O_7$	98	3.14	d10-BECdiprop	21.5	$[M+H]^+$	473	381/101	16/22	60
MPNLprop	**Methylprednisolone 21-propionate**	TRC	138804-88-3	$C_{25}H_{34}O_6$	-	2.70	d5-CLOprop	20.6	$[M+H]^+$	431	339/253	15/32	50
MOM	**Mometasone**	TRC	105102-22-5	$C_{22}H_{28}Cl_2O_4$	-	3.50	d6-LNG	20.8	$[M+H]^+$ /$[M+HCOO]^-$	427 /471	373 /435	16 /-15	60 /-30
MOMfur	**Mometasone 17-furoate**	SA	83919-23-7	$C_{27}H_{30}Cl_2O_6$	63	5.06	d5-FLUprop	21.9	$[M+H]^+$	521	355/373	23/17	50
PNL	**Prednisolone**	SA	50-24-8	$C_{21}H_{28}O_5$	3175	1.27	d8-PNL	15.3	$[M+HCOO]^-$	405	359/329	-15/-23	-10
PNS	**Prednisone**	SA	53-03-2	$C_{21}H_{26}O_5$	354	1.66	d8-PNL	14.5	$[M+HCOO]^-$	403	357/327	-12/-19	-20
TRIact	**Triamcinolone acetonide**	SA	76-25-5	$C_{24}H_{31}FO_6$	1155	1.94	13C3-TRIact	17.6	$[M+H]^+$	435	415/397	14/20	80
TRIact-m1	**6ß-Hydroxy triamcinolone acetonide**	TRC	3869-32-7	$C_{24}H_{31}FO_7$	-	0.71	13C3-TRIact	13.4	$[M+H]^+$	451	387/329	13/20	90
Mineralocorticoids (MC)													
CAN	**Canrenone**	SA	976-71-6	$C_{22}H_{28}O_3$	-	3.60	d6-CAN	20.8	$[M+H]^+$	341	107/187	35/32	110
CAN-m1	**11α-Hydroxy canrenone**	TRC	192569-17-8	$C_{22}H_{28}O_4$	-	2.29	d4-E1	17.0	$[M-H]^-$	355	311/267	-20/-25	-130
FLC	**Fludrocortisone**	TRC	127-31-1	$C_{21}H_{29}FO_5$	-	1.32	d5-DMS	14.7	$[M+H]^+$	381	361/343	28/28	130
FLCac	**Fludrocortisone 21-acetate**	SA	514-36-3	$C_{23}H_{31}FO_6$	0.4	1.76	13C3-TRIact	18.9	$[M+H^+]^+$	423	343/325	30/31	120
SPL	**Spironolactone**	SA	52-01-7	$C_{24}H_{32}O_4S$	9150	3.64	d6-CAN	20.8	$[M+H]^+$	417	341	20	40

SPL-m1	**7α-Thiomethyl spirono-lactone**	TRC	38753-77-4	$C_{23}H_{32}O_3S$	-	4.18	d7-SPL-m1	20.9	$[M+H]^+$	389	341/323	25/23	110
Surrogates													
Internal standard mix 1 (IS-mix 1)													
d8-BDN	**Budesonide-d8**	TRC	-	$C_{25}H_{34}O_6$	-	-	-	19.8 (19.9)	$[M+H]^+$	439	323	19	40
d6-CAN	**Canrenone-d6**	TRC	-	$C_{22}H_{22}D_6O_3$	-	-	-	20.7	$[M+H]^+$	347	107	37	110
d5-CLOprop	**Clobetasol 17-propionate-d5**	TRC	-	$C_{25}H_{27}D_5ClFO_5$	-	-	-	21.7	$[M+H]^+$	472	373	17	70
d3-CYPac	**Cyproterone acetate-d3**	TRC	-	$C_{24}H_{26}D_3ClO_4$	-	-	-	21.9	$[M+H]^+$	420	357	25	100
d5-DMS	**Dexamethasone-d5**	TRC	-	$C_{22}H_{24}D_5FO_5$	-	-	-	16.6	$[M+HCOO]^-$ /$[M+H]^+$	442 /398	364 /378	-25 /17	-10 /70
d8-DIE	**Dienogest-d8**	TRC	-	$C_{20}H_{17}D_8NO_2$	-	-	-	17.4	$[M+H]^+$	320	167	38	160
13C3-DPN	**Drospirenone-13C3**	TRC	-	$C_{21}{}^{13}C_3H_{30}O_3$	-	-	-	20.6	$[M+H]^+$	370	97	35	100
d3-FMS	**Flumethasone-d3**	TRC	-	$C_{22}H_{25}D_3F_2O_5$	-	-	-	16.8	$[M+HCOO]^-$	458	382	-24	-30
d6-LNG	**Levonorgestrel-d6**	TRC	-	$C_{21}H_{22}D_6O_2$	-	-	-	20.8	$[M+H]^+$	319	251	25	120
d6-NES	**Norethisterone-d6**	TRC	-	$C_{20}H_{20}D_6O_2$	-	-	-	19.7	$[M+H]^+$	305	237	27	100
d8-PNL	**Prednisolone-d8**	TRC	-	$C_{21}H_{20}D_8O_5$	-	-	-	15.3	$[M+HCOO]^-$	413	367	-16	-10
d5-FLUprop	**Fluticasone 17-propionate-d5**	TRC	-	$C_{25}H_{26}D_5F_3O_5S$	-	-	-	21.7	$[M+H]^+$	506	313	20	80
13C3-TRIact	**Triamcinolone acetonide-13C3**	TRC	-	$C_{21}{}^{13}C_3H_{31}FO_6$	-	-	-	17.6	$[M+H]^+$	438	418	15	80
d7-SPL-m1	**7α-Thiomethyl spirono-lactone-d7**	TRC	-	$C_{23}H_{25}D_7O_3S$	-	-	-	20.8	$[M+H]^+$	396	348	25	110
d4-E1	**Estrone-d4**	SA	-	$C_{18}H_{18}D_4O_2$	-	-	-	20.2	$[M-H]^-$	273	147	-50	-100
Internal standard mix 2 (IS-mix 2)													
d10-BECdirop	**Beclomethasone 17, 21-diropionate-d10**	TRC	-	$C_{28}H_{27}D_{10}ClO_7$	-	-	-	22.3	$[M+H]^+$	531	319	25	30
d3-BMSac	**Betamethasone 21-acetate-d3**	TRC	-	$C_{24}H_{28}D_3FO_6$	-	-	-	19.6	$[M+H]^+$	438	418	12	50
d5-BMSprop	**Betamethasone 17-propionate-d5**	TRC	-	$C_{25}H_{28}D_5FO_6$	-	-	-	19.8	$[M+H]^+$	454	434	12	70
d10-BMSdiprop	**Betamethasone 17,21-dipropionate-d10**	TRC	-	$C_{28}H_{27}D_{10}FO_7$	-	-	-	22.0	$[M+H]^+$	515	416	17	50

Table A 15: Measured environmental samples, sampling dates, locations and capacities of waste water treatment plants

Abbreviation	Name/capacity	Sampling Date	Location
WWTP effluent samples			
WWTPeff 1	25,000 citizens (person equivalents not known)	17/05/23	Groß-Gerau (Hessia)
WWTPeff 2	24,500 citizens (person equivalents not known)	17/05/23	Bingen (RLP)
WWTPeff 3	26,487 person equivalents (size: 48,000 pe)	17/05/26	Schwelm (NRW)
WWTPeff 4	220,000 person equivalents (size: 320,000 pe)	17/03/14	Koblenz (RLP)
WWTPeff 5	105,000 m³/day wastewater (dry weather conditions)	17/05/30	Wandlitz (Branden-burg)
Rivers and streams			
SW-1a	Mühlenbach (upstream WWTP)	17/05/23	Groß-Gerau
SW-1b	Mühlenbach (downstream WWTP)	17/05/23	Groß-Gerau
SW-2a	River Nahe (upstream WWTP)	17/05/23	Bingen
SW-2b	River Nahe (downstream WWTP)	17/05/23	Bingen
SW-3a	Schwelme (upstream WWTP)	17/05/26	Schwelm
SW-3b	Schwelme (downstream WWTP, immedi-ately for entry in river Wupper)	17/05/26	Wuppertal
SW-4a	River Wupper (upstream entry Schwelme)	17/05/26	Wuppertal
SW-4b	River Wupper (downstream entry Schwelme)	17/05/26	Wuppertal
SW-5	Teltow canal	17/08/14	Berlin
SW-6	Landgraben (downstream industrial WWTP)	17/05/23	Weiterstadt
SW-7	River Neckar	17/05/23	Mannheim
SW-8	River Main	17/05/23	Wiesbaden
SW-9a	River Lahn (XX km)	17/05/23	Limburg a.d.Lahn
SW-9b	River Lahn (XX km)	17/05/24	Lahnstein
SW-10a	River Rhine (km 432)	17/05/23	Frankenthal
SW-10b	River Rhine (km 434)	17/05/23	Frankenthal
SW-10c	River Rhine (km 482)	17/05/23	Trebur
SW-10d	River Rhine (km 590)	17/03/06	Koblenz
SW-10e	River Rhine (km 590)	17/04/25	Koblenz
SW-10f	River Rhine (km 590)	17/06/01	Koblenz
SW-11	River Ahr	17/05/22	Sinzig
SW-12	River Rur	17/05/21	Kreuzau

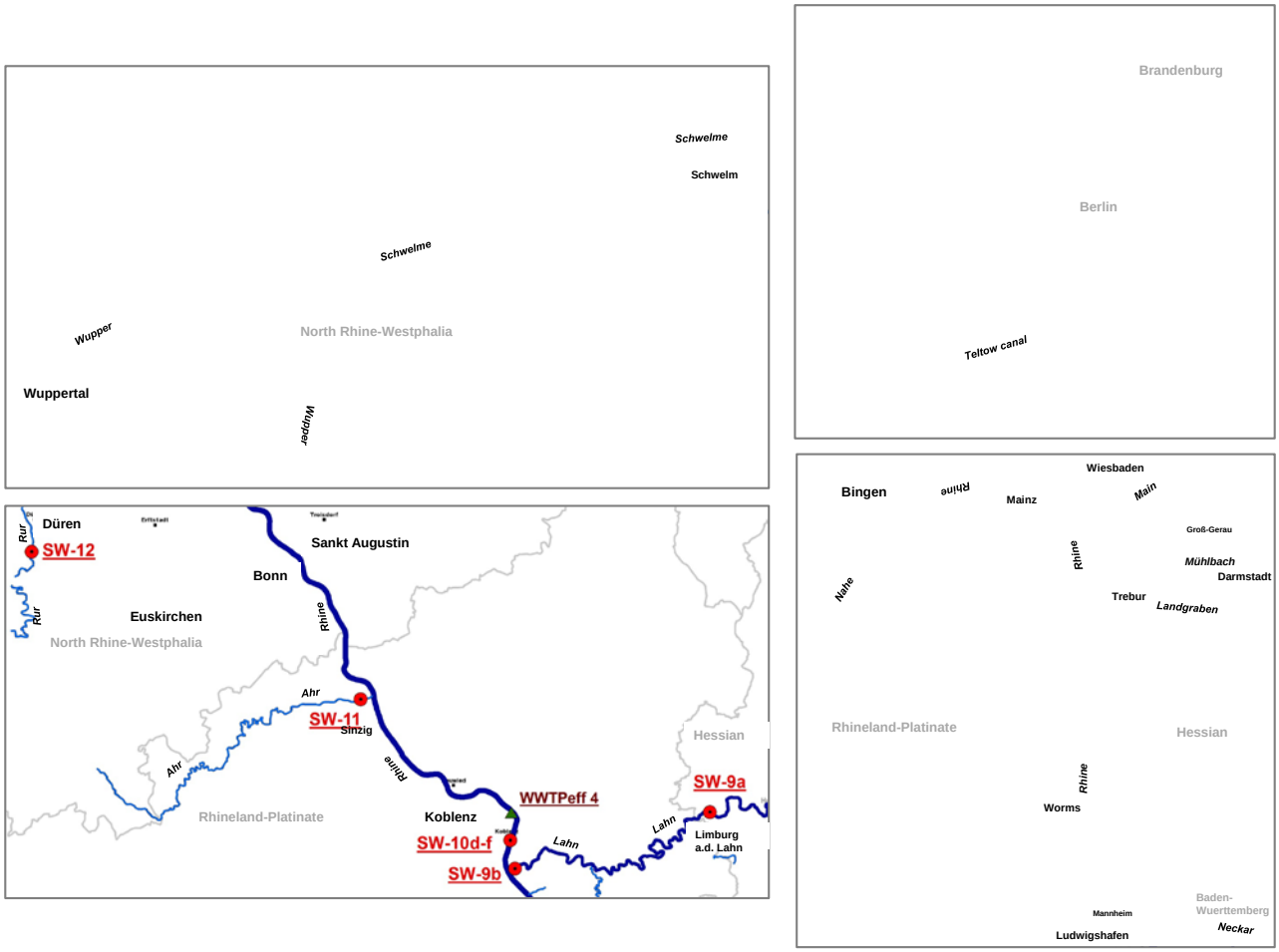


Figure A 2: Map sections of sampling locations

Table A 16: Properties of target steroid hormones. Dosage forms were assembled from pharma-bund.de. Application quantities prescribed in Germany 2014 were calculated based on the number of prescribed daily doses x defined daily doses.

Name	CAS	Appl. Quantity [kg]	Dosage Form	Therapeutic use
Glucocorticoids (GC)				
6ß-Hydroxy budesonide (BDN-m1)	88411-77-2			Metabolite of BDN
6β-Hydroxy dexamethasone (DMS-m1)	55879-87-3	-		Metabolite of DMS/DMSac
6β-Hydroxy triamcinolone acetonide (TRIact-m1)	3869-32-7	-	-	Metabolite of TRIact
Beclomethasone (BEC)	4419-39-0	-		Metabolite of BECdiprop
Beclomethasone 17-propionate (BECprop)	5534-18-9	-	-	Active metabolite of BECdiprop
Beclomethasone dipropionate (BECdiprop)	5534-09-8	158	Solution/powder for Inhalation, nasal spray	Treatment of lung and bronchial diseases, allergic disorders, OTC (nasal spray for seasonal rhinitis)
Betamethasone (BMS)	378-44-9	7	Tablet, solution, injection	Allergic disorders, treatment of rheumatic disorder, autoimmune diseases, veterinary medicine, metabolite of BMSdiprop/BMSac/BMSval
Betamethasone 17-propionate (BMSprop)	5534-13-4			Active metabolite of BMSdiprop
Betamethasone 17-valerate (BMSval)	2152-44-5	98	Ointment, cream, solution, foam	Treatment of dermatitis, allergic disorders, veterinary medicine (only topically administration)
Betamethasone 21-acetate (BMSac)	987-24-6	7	Injection	Treatment of rheumatic disorder, autoimmune diseases, veterinary medicine
Betamethasone dipropionate (BMSdiprop)	5593-20-4	116	Ointment, cream, gel, foam, transdermal patch, injection	Dermatitis, therapy of Morbus Crohn and Colitis ulcerosa, allergic disorders

Budesonide (BDN)	51333-22-3	354	Inhalation powder, spray, tablet, foam, ointment	Treatment of lung and bronchial diseases, allergic rhinitis, Morbus Crohn
Ciclesonide (CIC)	126544-47-6	1	Solution for Inhalation	Prodrug of CIC-m1, treatment of lung and bronchial diseases
Clobetasol (CLO)	25122-41-2	-	- Metabolite of CLOprop	
Clobetasol propionate (CLOprop)	25122-46-7	89	Cream, ointment, solution, foam, shampoo	Treatment of inflammatory and allergic skin diseases (only topical administration)
Cortisol (HCOR)	50-23-7	605	Injection, tablet, drops, ointment, spray	Natural hormone, replacement therapy in patients with adrenocortical insufficiency, treatment of rheumatic disorder, allergic conditions, dermatitis, veterinary medicine, active metabolite of COR
Cortisone (Cor)	53-06-5	-	-	Prodrug of HCOR, natural hormone
Desisobutyryl ciclesonide (CIC-m1)	161115-59-9	-	-	Active metabolite of CIC
Dexamethasone (DMS)	50-02-2	277	Tablet, solution, drops, injection, nasal spray, cream, intravitreal implant	Allergic disorders, treatment of rheumatic disorder, treatment of eye diseases, autoimmune diseases, veterinary medicine
Dexamethasone 21-acetate (PNL)	1177-87-3	3	Drops, injection	Allergic disorders, treatment of rheumatic disorder, autoimmune diseases, veterinary medicine
Diflucortolone valerate (DFCval)	59198-70-8	3	Ointment, cream	Treatment of inflammatory and allergic skin diseases (only topical administration)
Flumethasone (FMS)	2135-17-3	-	-	Metabolite of FMSpiv
Flumethasone 21-pivalate (FMSpiv)	2002-29-1	1	Cream, tincture, ointment	Treatment of inflammatory and allergic skin diseases, actinic dermatitis
Fluocinolone acetonide (FCNact)	67-73-2	12	Cream, ointment, intravitreal implant, drops, suppository	Treatment of inflammatory and allergic skin diseases

Fluorometholone (FML)	426-13-1	3	Eye drops	Therapy of inflammatory eye diseases
Fluticasone furoate (FLUfur)	397864-44-7	2	Nasal spray, powder for inhalation	Allergic disorders, treatment of lung and bronchial diseases
Fluticasone propionate (FLUprop)	80474-14-2	80	Inhalation powder, nasal spray, cream	Dermatitis, allergic disorders, treatment of lung and bronchial diseases, OTC drug (nasal spray for seasonal rhinitis)
Halcinonide (HAL)	3093-35-4	-	Cream, ointment, solution	No drug approval in Germany (but manufacturer of HAL in Germany), permitted in bordering countries, treatment of inflammatory and allergic skin diseases (only topically administration)
Halomethasone (HLM)	50629-82-8	1	Cream, ointment	Treatment of inflammatory and allergic skin diseases (only topical administration)
Methylprednisolone (MPNL)	83-43-2	157	Tablet, injection	Allergic conditions, Morbus Crohn, treatment of rheumatic disorder and multiple sclerosis, veterinary medicine, metabolite of MPNLacp
Methylprednisolone 21-propionate (MPNLprop)	138804-88-3	-	-	Metabolite of MPNLacp
Methylprednisolone aceponate (MPNLacp)	86401-95-8	98	Cream, ointment, injection	Allergic conditions, dermatitis, veterinary medicine
Mometasone (MOM)	105102-22-5	-	-	Metabolite of MOMfur
Mometasone furoate (MOMfur)	83919-23-7	63	Drops, nasal spray, powder for inhalation, cream, ointment	Therapy of rhinitis, asthma, treatment of inflammatory and allergic skin diseases, OTC (nasal spray for seasonal rhinitis), Veterinary medicine
Prednisolone (PNL)	50-24-8	3175	Cream, tincture, injection, tablet, ointment, suppository	Treatment of lung diseases, allergic disorders, Morbus Crohn, dermatitis, veterinary medicine, active metabolite of PNS
Prednisone (PNS)	53-03-2	354	Tablet, suppository	Prodrug of PNL, allergic disorders, Morbus Crohn, dermatitis

Triamcinolone acetonide (TRIact)	76-25-5	1155	Spray, drops, tablet, cream, ointment, injection, dental powder, nasal spray, tincture	Treatment of seasonal rhinitis, dermatitis, therapy of arthrosis and rheumatic disorder, veterinary medicine, OTC drug (as tablet)
Mineralocorticoids (MC)				
11α-Hydroxy canrenone (CAN-m1)	192569-17-8	-	-	Metabolite of SPL, treatment of high blood pressure, chronic heart failure, liver and kidney diseases, hormone therapy
7α-Thiomethyl spironolactone (SPL-m1)	38753-77-4	-	-	Metabolite of SPL, treatment of high blood pressure, chronic heart failure, liver and kidney diseases, hormone therapy
Canrenone (CAN)	976-71-6	9150 kg (Spironolactone)	Tablet, injection	Active metabolite of spironolactone, treatment of high blood pressure, chronic heart failure, liver and kidney diseases, hormone therapy
Fludrocortisone (FLC)	127-31-1	0.4 (all FLC derivatives)	Tablet	Active metabolite of FLCac
Fludrocortisone acetate (FLCac)	514-36-3	0.4 (all FLC derivatives)	Tablet, ear drops, solution, emulsion	Treatment of Addison disease, therapy of ear infections, veterinary medicine, (prodrug of FLC)
Progestogens (PG)				
17α-Hydroxy progesterone (HPG)	68-96-2	n.a.	Injection (as its caproate ester)	Natural hormone, prevention of preterm birth
6β-Hydroxy dienogest (DIE-m1)	n.a.	-	-	Metabolite of DIE
6β-Hydroxy progesterone acetate (MRPac-m1)	984-47-4	-	-	Metabolite of MRPac
Chlormadinone (CLM)	1961-77-9	-	-	Metabolite of CLMac
Chlormadinone acetate (CLMac)	302-22-7	99	Tablet, Injection	Hormonal contraception, hormone replacement therapy, treatment of gynecological disorders, veterinary medicine
Cyproterone (CYP)	2098-66-0	-	-	Metabolite of CYPac

Cyproterone acetate (CYPac)	427-51-0	99	Tablet, injection	Hormonal contraception, hormone replacement therapy, hormone therapy, treatment of dermatological conditions, treatment of cancer
Dienogest (DIE)	65928-58-7	278	Tablet	Hormonal contraception, hormone replacement, treatment of gynecological disorders and dermatological conditions
Drospirenone (DPN)	67392-87-4	61	Tablet	Hormonal contraception, treatment of dermatological conditions
Etonogestrel (ETG)	54048-10-1	0.4	Implant, intrauterine devices	Long-term contraception
Gestodene (GES)	60282-87-3	n.a.	Tablet, transdermal patch	Hormonal contraception
Levonorgestrel (LNG)	797-63-7	17	Tablet, transdermal patch, implant, intrauterine devices	Hormonal contraception, emergency contraception, hormone replacement therapy
Medroxy progesterone (MRP)	520-85-4	-	-	Metabolite of MRPac
Medroxy progesterone acetate (MRPac)	71-58-9	570	Injection, tablet	Long-term hormonal contraception, hormone replacement therapy, treatment of cancer, veterinary medicine
Megestrol (MEG)	3562-63-8	-	-	Metabolite of MEGac
Megestrol acetate (MEGac)	595-33-5	n.a.	Tablet	Treatment of cancer, veterinary medicine
Norethisterone (NES)	68-22-4	21 (all NES derivatives)	Tablet, injection (in part as enantate ester)	Hormonal contraception, hormone replacement therapy, treatment of gynecological disorders (Metabolite of NESac)
Norethisterone acetate (NESac)	51-98-9	21 (all NES derivatives)	Tablet, injection, transdermal patch	Hormonal contraception, hormone replacement therapy, treatment of gynecological disorders

Table A 17: Concentrations of target analytes in German WWTP effluents. LOD and LOQ calculations were based on a signal-to-noise ratio of 3 (LOD) and 10 (LOQ) either using the background concentration or a total spike amount in the smoothed (smoothing *factor: 2.0) chromatograms of environmental samples. (< = below detection limit, <LOQ= above detection limit, below quantification limit)*

	Concentration [ng/L] WWTP effluent					
Substance	**1**	**2**	**3**	**4**	**5**	**LOD/LOQ**
Mineralocorticoids (MC)						
11α-Hydroxy canrenone	<LOQ	<	<	<	<	0.5 / 3.0
7α-Thiomethyl spironolactone	0.2	1.2	1.5	3.8	2.0	0.05 / 0.1
Canrenone	4.5	3.7	10	19	8.0	0.4 / 1.4
Fludrocortisone	<	<	<	<	<	0.5 / 0.8
Fludrocortisone acetate	<	<	<	<	<	0.5 / 1.5
Glucocorticoids (GC)						
6ß-Hydroxy budesonide	<	<	<	<	<	0.2 / 0.5
6ß-Hydroxy dexamethasone	<	<	<	<	<	0.07 / 0.2
6ß-Hydroxy triamcinolone acetonide	1.2	1.7	6.9	2.3	2.2	0.06 / 0.2
6α-Methylprednisolone	<LOQ	<	0.1	1.0	0.2	0.02 / 0.06
6α-Methylprednisolone aceponate	<	<	<	<	<	0.3 / 0.5
6α-Methylprednisolone propionate	1.4	<LOQ	2.4	0.5	4.2	0.2 / 0.5
Beclomethasone	<	<LOQ	<LOQ	<	<	0.02 / 0.07
Beclomethasone dipropionate	<	<	<	<	<	0.1 / 0.5
Beclomethasone propionate	<LOQ	<LOQ	<LOQ	<	<	0.1 / 0.3
Betamethasone	0.6	0.4	0.05	0.2	0.6	0.01 / 0.05
Betamethasone 21-acetate	<	<	<	<	<	0.05 / 0.2
Betamethasone dipropionate	<	<	<	<	<	0.08 / 0.3

Betamethasone propionate	1.1	1.5	1.2	3.6	0.3	0.08 / 0.2
Betamethasone valerate	1.3	2.5	1.1	2.2	1.2	0.08 / 0.3
Budesonide	<	<	1.2	2.0	<	0.5 / 1.0
Ciclesonide	<	<	<	<	<	0.06 / 0.3
Clobetasol	<	<	<	<	<	0.2 / 0.5
Clobetasol propionate	0.5	0.8	2.1	4.0	5.4	0.08 / 0.3
Cortisol (Hydrocortisone)	0.9	1.4	1.2	2.8	0.9	0.06 / 0.2
Cortisone	0.2	0.3	0.4	0.9	0.2	0.1 / 0.2
Desisobutyryl ciclesonide	<	<	<	<	<	0.5 / 1.0
Dexamethasone	<	<	<	<	<	0.05 / 0.1
Dexamethasone 21-acetate	<	<	<	<	<	0.3 / 0.5
Diflucortolone valerate	<	<	<	<	<	0.02 / 0.05
Flumetasone	<	<	<	<	<	0.05 / 0.1
Flumetasone 21-pivalate	<	<	<	<	<	0.04 / 0.1
Fluocinolone acetonide	0.1	0.1	0.1	0.2	0.2	0.03 / 0.1
Fluorometholone	<	<	<	<	<	0.05 / 0.3
Fluticasone 17-furoate	<LOQ	<	<	<	<LOQ	0.05 / 0.2
Fluticasone 17-propionate	<LOQ	0.1	0.5	1.0	0.9	0.05 / 0.1
Halcinonide	<	<	<	<	<	0.02 / 0.3
Halomethasone	<	<	<	<	<	0.1 / 0.5
Mometasone	<	<	<	<	<	1.0 / 2.0
Mometasone 17-furoate	0.8	1.2	1.7	2.2	1.4	0.08 / 0.3
Prednisolone	<LOQ	<LOQ	0.4	0.6	<LOQ	0.06 / 0.2
Prednisone	<LOQ	<LOQ	0.2	0.4	<LOQ	0.06 / 0.2
Triamcinolone acetonide	6.3	5.5	17	11	28	0.1 / 0.5

Progestogens (PG)						
17α-Hydroxy progesterone	1.1	0.7	0.7	1.0	1.3	0.3 / 0.7
6ß-Hydroxy dienogest	<LOQ	0.6	0.6	0.6	0.9	0.2 / 0.4
6ß-Hydroxy medroxy progesterone acetate	<	<	<	<	<	0.2 / 0.5
Chlormadinone	<	<<	<	<	<	1.5 / 5.0
Chlormadinone acetate	<	<LOQ	<LOQ	<LOQ	<	0.1 / 0.3
Cyproterone	<	<	<	<	<	0.5 / 1.0
Cyproterone acetate	0.8	1.7	2.9	3.7	2.3	0.3 / 0.8
Dienogest	3.3	1.3	4.4	4.3	1.4	0.2 / 0.3
Drospirenone	<	<	<	<	<	0.5 / 1.0
Etonogestrel	<	<	<	<	<	0.5 / 2.0
Gestodene	<	<	<	<	<	1.0 / 2.5
Levonorgestrel	<	<LOQ	<	<	<	0.3 / 1.0
Medroxy progesterone	<	<	<	<	<	0.08 / 0.3
Medroxy progesterone acetate	<LOQ	<LOQ	<LOQ	<	<	0.08 / 0.3
Megestrol	<	<	<	<	<	0.5 / 1.0
Megestrol acetate	<	<LOQ	<	<	<	0.06 / 0.3
Norethisterone	<	<	<	<	<	1.0 / 1.5
Norethisterone acetate	<	<	<	<	<	0.5 / 1.0

Table A 18: Concentrations of target analytes in German rivers/ streams. LOD and LOQ calculations were based on a signal-to-noise ratio of 3 (LOD) and 10 (LOQ) either using the background concentration or a total spike amount in the smoothed smoothing *factor: 2.0) chromatograms of environmental samples. (< = below detection limit, <LOQ= above detection limit, below quantification limit)*

	Concentration [ng/L] Surface water											
Substance	**SW-1a**	**SW-1b**	**SW-2a**	**SW-2b**	**SW-3a**	**SW-3b**	**SW-4a**	**SW-4b**	**SW-5**	**SW-6**	**SW-7**	**LOD/LOQ**
Mineralocorticoids (MC)												
11α-Hydroxy canrenone	<	0.4	<	<	<	<	<	<	<	<	<	0.1 / 0.3
7α-Thiomethyl spironolactone	<	0.1	0.2	0.3	<	1.3	0.03	0.2	0.6	0.2	0.07	0.01 / 0.03
Canrenone	<	3.0	1.6	1.6	<	8.3	0.5	1.2	2.9	1.8	0.6	0.08 / 0.2
Fludrocortisone	<	<	<	<	<	<	<	<	<	<	<	0.05 / 0.3
Fludrocortisone acetate	<	<	<	<	<	<	<	<	<	<	<	0.3 / 0.5
Glucocorticoids (GC)												
6ß-Hydroxy budesonide	<	<	<	<	<	<	<	<	<	<LOQ	<	0.05 / 0.1
6ß-Hydroxy dexamethasone	<	<	<	<	<	<	<	<	<	<	<	0.01 / 0.02
6ß-Hydroxy triamcinolone acetonide	<	0.9	0.1	0.2	<	5.1	<LOQ	0.6	1.2	0.8	<	0.03 / 0.05
6α-Methylprednisolone	<	<LOQ	<LOQ	<LOQ	<	0.2	<LOQ	0.05	0.2	<LOQ	<LOQ	0.01 / 0.05
6α-Methylprednisolone aceponate	<	<	<	<	<	<	<	<	<	<	<	0.02 / 0.1
6α-Methylprednisolone propionate	<	0.9	<	<	<	1.3	<	<LOQ	0.9	0.6	<	0.06 / 0.2
Beclomethasone	<	<	<	<	<	<	<	<	0.07	<	<	0.02 / 0.05
Beclomethasone dipropionate	<	<	<	<	<	<	<	<	<	<	<	0.05 / 0.2
Beclomethasone propionate	<	<LOQ	<	<	<	<	<	<	<	<	<	0.1 / 0.3
Betamethasone	<	0.5	0.2	0.2	<	0.4	<LOQ	0.05	1.0	0.3	0.1	0.02 / 0.05
Betamethasone 21-acetate	<	<	<	<	<	<	<	<	<	<	<	0.03 / 0.1

Betamethasone dipropionate	<	<	<	<	<	<LOQ	<	<	<	<	<	0.02 / 0.2
Betamethasone propionate	<	0.9	0.05	0.2	<	0.6	<LOQ	0.07	0.4	1.2	<LOQ	0.02 / 0.05
Betamethasone valerate	<	0.9	<LOQ	0.2	<	0.7	<	<LOQ	0.2	1.3	<	0.03 / 0.2
Budesonide	<	<	<	<	<	0.7	<	<LOQ	<	<LOQ	<	0.3 / 0.5
Ciclesonide	<	<	<	<	<	<	<	<	<	<	<	0.03 / 0.05
Clobetasol	<	<	<	<	<	<	<	<	<	<	<	0.05 / 0.3
Clobetasol propionate	<	0.4	0.1	0.2	<	3.4	<LOQ	0.3	1.7	0.2	0.05	0.02 / 0.05
Cortisol (Hydrocortisone)	0.2	0.7	1.3	1.3	0.2	1.3	0.3	0.4	0.2	1.0	0.6	0.02 / 0.08
Cortisone	0.1	0.2	0.3	0.4	0.2	0.7	0.2	0.3	0.08	0.2	0.6	0.01 / 0.02
Desisobutyryl ciclesonide	<	<	<	<	<	<	<	<	<	<	<	0.1 / 0.3
Dexamethasone	<	<	<	<	<	<	<	<	<	<LOQ	<	0.02 / 0.05
Dexamethasone 21-acetate	<	<	<	<	<	<	<	<	<	<	<	0.02 / 0.07
Diflucortolone valerate	<	<	<	<	<	<	<	<	<	<	<	0.01 / 0.02
Flumetasone	<	<	<	<	<	<	<	<	<	<	<	0.02 / 0.05
Flumetasone 21-pivalate	<	<	<	<	<	<	<	<	<	<LOQ	<	0.02 / 0.05
Fluocinolone acetonide	<	0.09	<LOQ	<LOQ	<	0.1	<LOQ	<LOQ	0.09	0.1	<	0.02 / 0.05
Fluorometholone	<	<	<	<	<	<	<	<	<	<	<	0.02 / 0.03
Fluticasone 17-furoate	<	<LOQ	<	<	<	<	<	<	<	<	<	0.05 / 0.1
Fluticasone 17-propionate	<	<LOQ	<LOQ	<LOQ	<	0.4	<LOQ	<LOQ	0.3	0.2	<LOQ	0.05 / 0.1
Halcinonide	<	<	<	<	<	<	<	<	<	<	<	0.02 / 0.1
Halomethasone	<	<	<	<	<	<	<	<	<	<	<	0.05 / 0.3
Mometasone	<	<	<	<	<	<	<	<	<	<	<	0.3 / 0.5
Mometasone 17-furoate	<	0.6	<LOQ	<LOQ	<	1.0	<	<LOQ	0.2	0.8	<	0.05 / 0.2
Prednisolone	0.2	0.05	0.2	0.07	0.07	0.4	0.1	0.06	<LOQ	0.05	0.1	0.02 / 0.05
Prednisone	<	<LOQ	<LOQ	<LOQ	<	<LOQ	<	<LOQ	<	0.05	<	0.03 / 0.05

Triamcinolone acetonide	0.04	4.4	0.7	1.0	<	12	0.09	1.5	7.6	8.5	0.3	0.01 / 0.04
Progestogens (PG)												
17α-Hydroxy progesterone	<	0.6	<	<LOQ	<	<LOQ	<	<	<	0.6	<	0.3 / 0.5
6ß-Hydroxy dienogest	<	<	<	<	<	0.4	<	<	<	0.5	<	0.05 / 0.1
6ß-Hydroxy medroxy progester-one acetate	<	<	<	<	<	<	<	<	<	<	<	0.05 / 0.1
Chlormadinone	<	<	<	<	<	<	<	<	<	<	<	0.1 / 0.5
Chlormadinone acetate	<	<	<	<	<	0.1	<	<	<	<	<	0.05 / 0.1
Cyproterone	<	<	<	<	<	<	<	<	<	<	<	0.1 / 0.3
Cyproterone acetate	<	0.6	<LOQ	0.2	<	2.6	<	0.3	0.9	0.6	<	0.05 / 0.2
Dienogest	<	2.3	0.08	0.2	<	2.0	<	0.3	<	0.1	0.05	0.02 / 0.05
Drospirenone	<	<	<	<	<	<	<	<	<	<	<	0.3 / 0.5
Etonogestrel	<	<	<	<	<	<	<	<	<	<	<	0.3 / 0.5
Gestodene	<	<	<	<	<	<	<	<	<	<	<	0.3 / 0.5
Levonorgestrel	<	<	<	<	<	0.5	<	<	<	0.7	<	0.05 / 0.3
Medroxy progesterone	<	<	<	<	<	<	<	<	<	<	<	0.05 / 0.1
Medroxy progesterone acetate	<	0.1	<LOQ	<	<	0.1	<	<	<	<	<	0.05 / 0.1
Megestrol	<	<	<	<	<	<	<	<	<	<	<	0.3 / 0.4
Megestrol acetate	<	<	<	<LOQ	<	<	<	<	<	<	<	0.05 / 0.2
Norethisterone	<	<	<	<	<	<	<	<	<	<LOQ	<	0.1 / 0.3
Norethisterone acetate	<	<	<	<	<	<	<	<	<	<	<	0.3 / 0.5

Table A 19: Concentrations of target analytes in German rivers/ streams. LOD and LOQ calculations were based on a signal-to-noise ratio of 3 (LOD) and 10 (LOQ) either using the background concentration or a total spike amount in the smoothed smoothing *factor: 2.0) chromatograms of environmental samples. (< = below detection limit, <LOQ= above detection limit, below quantification limit)*

Substance	Concentration [ng/L] Surface water											
	SW-8	SW-9a	SW-9b	SW-10a	SW-10b	SW-10c	SW-10d	SW-10e	SW-10f	SW-11	SW-12	LOD/LOQ
Mineralocorticoids (MC)												
11α-Hydroxy canrenone	<	<	<	<	<	<	<	<	<	<	<	0.1 / 0.3
7α-Thiomethyl spironolactone	0.08	0.3	0.2	<LOQ	<LOQ	<LOQ	0.05	0.03	<LOQ	<LOQ	<LOQ	0.01 / 0.03
Canrenone	0.4	0.8	1.0	<LOQ	0.2	0.2	0.5	0.2	<LOQ	0.2	0.2	0.08 / 0.2
Fludrocortisone	<	<	<	<	<	<	<	<	<	<	<	0.05 / 0.3
Fludrocortisone acetate	<	<	<	<	<	<	<	<	<	<	<	0.3 / 0.5
Glucocorticoids (GC)												
6ß-Hydroxy budesonide	<	<	<	<	<	<	<	<	<	<	<	0.05 / 0.1
6ß-Hydroxy dexamethasone	<	<	<	<	<	<	<	<	<	<	<	0.01 / 0.02
6ß-Hydroxy triamcinolone acetonide	0.1	0.08	0.08		<LOQ	<LOQ	0.05		<LOQ			0.03 / 0.05
6α-Methylprednisolone	<LOQ	<LOQ	<LOQ	<	<	<	<LOQ	<	<	<	<	0.01 / 0.05
6α-Methylprednisolone aceponate	<	<	<	<	<	<	<	<	<	<	<	0.02 / 0.1
6α-Methylprednisolone propionate	<	<	<	<	<	<	<	<	<	<	<	0.06 / 0.2
Beclomethasone	<	<	<LOQ	<	<	<	<	<	<	<	<	0.02 / 0.05
Beclomethasone dipropionate	<	<	<	<	<	<	<	<	<	<	<	0.05 / 0.2
Beclomethasone propionate	<	<	<	<	<	<	<	<	<	<	<	0.1 / 0.3
Betamethasone	0.1	0.1	0.09	<	<	<	<	<LOQ	<	<LOQ	<LOQ	0.02 / 0.05
Betamethasone 21-acetate	<	<	<	<	<	<	<	<	<	<	<	0.03 / 0.1

Betamethasone dipropionate	<	<	<	<	<	<	<	<	<	<	<	0.02 / 0.2
Betamethasone propionate	<	0.07	<LOQ	<	<	<LOQ	0.09	<LOQ	<	<	<	0.02 / 0.05
Betamethasone valerate	<LOQ	<LOQ	<LOQ	<	<	<	<LOQ	<	<	<	<	0.03 / 0.2
Budesonide	<	<	<	<	<	<	<	<	<	<	<	0.3 / 0.5
Ciclesonide	<	<	<	<	<	<	<	<	<	<	<	0.03 / 0.05
Clobetasol	<	<	<	<	<	<	<	<	<	<	<	0.05 / 0.3
Clobetasol propionate	0.1	0.1	0.09	<LOQ	<	<	0.06	<LOQ	<	<	<	0.02 / 0.05
Cortisol (Hydrocortisone)	0.7	1.3	1.2	0.3	0.2	0.5	0.3	0.1	0.2	0.7	0.2	0.02 / 0.08
Cortisone	0.7	1.0	0.8	0.3	0.1	0.4	0.1	0.02	0.06	0.2	0.4	0.01 / 0.02
Desisobutyryl ciclesonide	<	<	<	<	<	<	<	<	<	<	<	0.1 / 0.3
Dexamethasone	<	<	<LOQ	<	<	<	<	<	<	<	<	0.02 / 0.05
Dexamethasone 21-acetate	<	<	<	<	<	<	<	<	<	<	<	0.02 / 0.07
Diflucortolone valerate	<	<	<	<	<	<	<	<	<	<	<	0.01 / 0.02
Flumetasone	<	<	<	<	<	<	<	<	<	<	<	0.02 / 0.05
Flumetasone 21-pivalate	<	0.05	<	<	<	<	<	<	<	<	<	0.02 / 0.05
Fluocinolone acetonide	<	<LOQ	<LOQ	<	<	<	<	<	<	<	<	0.02 / 0.05
Fluorometholone	<	<	<	<	<	<	<	<	<	<	<	0.02 / 0.03
Fluticasone 17-furoate	<	<	<	<	<	<	<	<	<	<	<	0.05 / 0.1
Fluticasone 17-propionate	<	<LOQ	<	<	<	<	<LOQ	<	<	<	<	0.05 / 0.1
Halcinonide	<	<	<	<	<	<	<	<	<	<	<	0.02 / 0.1
Halomethasone	<	<	<	<	<	<	<	<	<	<	<	0.05 / 0.3
Mometasone	<	<	<	<	<	<	<	<	<	<	<	0.3 / 0.5
Mometasone 17-furoate	<	<LOQ	<	<	<	<	<	<	<	<	<	0.05 / 0.2
Prednisolone	0.07	<LOQ	0.05	<LOQ	<LOQ	0.05	0.08	0.05	<LOQ	0.05	0.09	0.02 / 0.05
Prednisone	<LOQ	0.05	<LOQ	<	<	<	<LOQ	<	<	<	<	0.03 / 0.05

Triamcinolone acetonide	0.6	0.3	0.3	<LOQ	0.06	0.06	0.3	0.07	0.08	0.05	0.1	0.01 / 0.05
Progestogens (PG)												
17α-Hydroxy progesterone	<	<	<	<	<	<	<	<	<	<	<	0.3 / 0.5
6ß-Hydroxy dienogest	<	<	<	<	<	<	<	<	<	<	<	0.05 / 0.1
6ß-Hydroxy medroxy progesterone acetate	<	<	<	<	<	<	<	<	<	<	<	0.05 / 0.1
Chlormadinone	<	<	<	<	<	<	<	<	<	<	<	0.1 / 0.5
Chlormadinone acetate	<	<	<	<	<	<	<	<	<	<	<	0.05 / 0.1
Cyproterone	<	<	<	<	<	<	<	<	<	<	<	0.1 / 0.3
Cyproterone acetate	<	<	<LOQ	<	<	<	<	<	<	<	<	0.05 / 0.2
Dienogest	0.05	0.09	0.06	<	<	<	<LOQ	<LOQ	<	<	<	0.02 / 0.05
Drospirenone	<	<	<	<	<	<	<	<	<	<	<	0.3 / 0.5
Etonogestrel	<	<	<	<	<	<	<	<	<	<	<	0.3 / 0.5
Gestodene	<	<	<	<	<	<	<	<	<	<	<	0.3 / 0.5
Levonorgestrel	<	<	<	<	<	<	<	<	<	<	<	0.05 / 0.3
Medroxy progesterone	<	<	<	<	<	<	<	<	<	<	<	0.05 / 0.1
Medroxy progesterone acetate	<	<LOQ	<	<	<	<	<	<	<	<	<	0.05 / 0.1
Megestrol	<	<	<	<	<	<	<	<	<	<	<	0.3 / 0.4
Megestrol acetate	<	<	<LOQ	<	<	<	<	<	<	<	<	0.05 / 0.2
Norethisterone	<	<	<	<	<	<	<	<	<	<	<	0.1 / 0.3
Norethisterone acetate	<	<	<	<	<	<	<	<	<	<	<	0.3 / 0.5

Table A 20: Recovery rates and reproducibility (expressed as 95%-confidence intervals) for the target steroid hormones in surface water at concentration levels 0.05, 0.25, 0.5 and 5 ng/L and WWTP effluent at concentration levels 0.5 ng/L, 1.0 ng/L, 10 ng/L and 50 ng/L. When the initial concentrations of the analytes were higher than the spike level, the recoveries were not determined. (n.d.= not determined, <LOD= below limit of detection)

Abbreviation	Substance	Surface water (1 L river Rhine) Recovery [%], (n=4)				WWTP effluent (0.5 L WWTP 4) Recovery [%], (n=4)			
		c=0.05 ng/L	c=0.25 ng/L	c=0.5 ng/L	c=5 ng/L	c=0.5 ng/L	c=1.0 ng/L	c=10 ng/L	c=50 ng/L
Mineralocorticoids (MC)									
CAN	Canrenone	-	102±8	107±8	116±11	-	-	-	101±4
CAN-m1	11α-Hydroxy canrenone	<LOD	89±13	80±16	80±5	<LOD	78±9	80±6	83±8
FLC	Fludrocortisone	86±32	86±23	94±17	92±10	73±16	71±7	76±5	75±8
FLCac	Fludrocortisone acetate	<LOD	87±11	93±6	85±10	85±9	99±8	103±7	100±7
SPL-m1	7α-Thiomethyl spironolactone	-	95±7	98±8	105±9	-	-	111±12	108±10
Glucocorticoids (GC)									
BDN	Budesonide	<LOD	108±23	101±10	104±3	-	-	93±9	99±4
BDN-m1	6ß-Hydroxy budesonide	102±17	102±13	101±10	102±18	99±9	100±10	101±5	90±8
CIC	Ciclesonide	71±10	85±14	73±8	81±19	62±4	65±5	74±11	77±6
CIC-m1	Desisobutyryl ciclesonide	<LOD	99±8	88±11	98±10	77±21	97±7	107±11	107±9
CLO	Clobetasol	109±13	102±21	93±16	105±21	100±5	95±11	105±9	107±8
CLOprop	Clobetasol propionate	-	97±9	101±8	106±6	-	-	109±13	115±11
DFCval	Diflucortolone valerate	93±12	95±23	89±13	87±13	93±12	98±15	102±5	101±3
DMS-m1	6ß-Hydroxy dexamethasone	75±9	99±26	99±20	101±14	88±6	100±17	95±16	90±6
FCNact	Fluocinolone acetonide	107±13	97±2	95±5	96±11	96±10	95±6	101±3	91±4
FLM	Fluorometholone	103±14	99±6	91±7	92±6	92±6	86±9	88±3	88±6
FLUfur	Fluticasone 17-furoate	106±26	93±12	89±8	97±10	101±11	105±11	98±5	104±7

FLUprop	Fluticasone 17-propionate	98±24	94±12	95±10	104±7	-	97±17	107±8	105±7
FMS	Flumetasone	103±13	102±4	101±8	108±10	109±4	106±8	112±9	104±8
FMSpiv	Flumetasone pivalate	103±10	106±6	99±14	101±5	101±6	102±6	104±4	102±10
MOM	Mometasone	<LOD	<LOD	69±19	93±8	<LOD	89±14	97±9	105±10
MOMfur	Mometasone 17-furoate	111±28	97±8	95±7	92±13	-	-	114±11	111±14
TRIact	Triamcinolone acetonide	-	-	104±11	110±12	-	-	-	102±7
TRIact-m1	6ß-Hydroxy triamcinolone acetonide	-	101±5	104±7	108±9	-	-	105±6	93±6
Progestogens (PG)									
DIE	Dienogest	106±14	106±5	103±17	101±12	-	-	97±13	107±9
DIE-m1	6β-Hydroxy dienogest	108±17	99±17	88±21	93±16	-	83±5	83±6	85±5
NES	Norethisterone	<LOD	101±19	97±16	101±13	<LOD	97±5	103±15	105±9
NESac	Norethisterone acetate	<LOD	93±11	90±10	91±7	102±10	96±7	99±4	107±1
DPN	Drospirenone	<LOD	98±17	94±21	99±7	100±14	107±7	107±4	103±9
ETG	Etonogestrel	<LOD	85±4	93±15	92±13	<LOD	95±19	92±9	95±10
GES	Gestodene	<LOD	106±29	109±18	122±17	<LOD	<LOD	86±11	99±9
CYP	Cyproterone	<LOD	93±15	94±14	104±11	101±22	99±17	103±5	107±11
CYPac	Cyproterone acetate	-	97±10	110±13	113±3	-	-	105±9	108±7
CLM	Chlormadinone	<LOD	96±3	91±8	89±5	<LOD	97±21	89±7	97±9
CLMac	Chlormadinone acetate	122±22	99±6	96±12	111±11	90±11	86±13	100±10	106±7
LNG	Levonorgestrel	<LOD	81±12	80±18	96±11	95±8	92±23	89±8	98±7
MRP	Medroxy progesterone	77±35	88±16	86±17	92±4	110±11	95±6	97±9	103±5
MRPac	Medroxy progesterone acetate	101±24	91±8	88±10	97±3	84±8	78±6	88±9	90±4
MRPac-m1	6ß-Hydroxy medroxy progesterone acetate	115±16	98±18	89±15	90±8	92±12	95±11	91±2	88±3
MEG	Megestrol	<LOD	77±10	90±21	107±10	89±19	98±10	87±6	98±5

MEGac	Megestrol acetate	89±24	96±8	95±6	98±4	99±8	92±10	87±7	93±9
HPG	17α-Hydroxy progesterone	<LOD	103±10	104±2	95±15	-	97±13	106±5	121±5