METHOD DEVELOPMENT FOR THE QUANTIFICATION OF PHARMACEUTICALS IN AQUEOUS ENVIRONMENTAL MATRICES

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Summary

As a consequence of the world population increase and the resulting water scarcity, water quality is the object of growing attention. In that context, organic anthropogenic molecules — often defined as micropollutants— represent a threat for water resources. Among them, pharmaceuticals are the object of particular concerns due to their permanent discharge, their increasing consumption and their effect-based structures. Pharmaceuticals are mainly introduced in the environment via wastewater treatment plants (WWTPs), along with their metabolites and the on-site formed transformation products (TPs). Once in the aquatic environment, they partition between the different environmental compartments in particular the aqueous phase, suspended particulate matter (SPM) and biota.
In the last decades, pharmaceuticals have been widely investigated in the water phase. However, extreme polar pharmaceuticals have rarely been monitored due to the lack of robust analytical methods. Moreover, metabolites and TPs have seldom been included in routine analysis methods although their environmental relevance is proven. Furthermore, pharmaceuticals have been only sporadically investigated in SPM and biota and adequate multi-residue methods are lacking to obtain comprehensive results about their occurrence in these matrices. This thesis endeavors to cover these gaps of knowledge by the development of generic multi-residue methods for pharmaceuticals determination in the water phase, SPM and biota and to evaluate the occurrence and partition of pharmaceuticals into these compartments. For a complete overview, a particular focus was laid on extreme polar pharmaceuticals, pharmaceutical metabolites and TPs. In total, three innovative multi-residue methods were developed, they include analytes covering a broad range of physico-chemical properties.

First, a reliable multi-residue method was developed for the analysis of extreme polar pharmaceuticals, metabolites and TPs dissolved in water. The selected analytes covered a significant range of elevated polarity and the method would be easily expendable to further analytes. This versatility could be achieved by the utilization of freeze-drying as sample preparation and zwitterionic hydrophilic interaction liquid chromatography (HILIC) in gradient elution mode. The suitability of HILIC chromatography to simultaneously quantify a large range of micropollutants in aqueous environmental samples was thoroughly studied. Several limitations were pointed out: a very complex and time-consuming method development, a very high sensitivity with regards to modification of the acetonitrile to water ratio in the eluent or the diluent and high positive matrix effects for certain analytes. However, these limitations can be overcome by the utilization of a precise protocol and appropriate labeled internal standards. They are overmatched by the benefits of HILIC which permits the chromatographic separation of extreme polar micropollutants. Investigation of environmental samples showed elevated concentrations of the analytes in the water phase. In particular, gabapentin, metformin, guanylurea and oxypurinol were measured at concentrations in the µg/L range in surface water.

Subsequently, a reliable multi-residue method was established for the determination of 57 pharmaceuticals, 47 metabolites and TPs sorbed to SPM down to the low ng/g range. This method was conceived to cover a large range of polarity in particular with the inclusion of extreme polar pharmaceuticals. The extraction procedure was based on pressurized liquid extraction (PLE) followed by a clean-up via solvent exchange and detection via direct injection-reversed-phase LC-MS/MS and freeze-drying-HILIC-MS/MS. Pharmaceutical sorption was examined using laboratory experiments. Derived distribution coefficients K_d varied by five orders of magnitude among the analytes

and confirmed a high sorption potential for positively charged and nonpolar pharmaceuticals. The occurrence of pharmaceuticals in German rivers SPM was evaluated by the investigation of annual composite SPM samples taken at four sites at the river Rhine and one site at the river Saar between the years 2005 and 2015. It revealed the ubiquitous presence of pharmaceuticals sorbed to SPM in these rivers. In particular, positively charged analytes, even very polar and nonpolar pharmaceuticals showed appreciable concentrations. For many pharmaceuticals, a distinct correlation was observed between the annual quantities consumed in Germany and the concentrations measured in SPM. Studies of composite SPM spatial distribution permitted to get hints about specific industrial discharge by comparing the pollution pattern along the river. For the first time, these results showed the potential of SPM for the monitoring of positively charged and nonpolar pharmaceuticals in surface water.

Finally, a reliable and generic multi-residue method was developed to investigate 35 pharmaceuticals and 28 metabolites and TPs in fish plasma, fish liver and fish fillet. For this matrix, it was very challenging to develop an adequate clean-up allowing for the sufficient separation of the matrix disturbances from the analytes. In the final method, fish tissue extraction was performed by cell disruption followed by a non-discriminating clean-up based on silica gel solid-phase extraction (SPE) and restrictive access media (RAM) chromatography. Application of the developed method to the measurement of bream and carp tissues from German rivers revealed that even polar micropollutants such as pharmaceuticals are ubiquitously present in fish tissues. In total, 17 analytes were detected for the first time in fish tissues, including 10 metabolites/TPs. The importance of monitoring metabolites and TPs in fish tissues was confirmed with their detection at similar concentrations as their parents. Liver and fillet were shown to be appropriate for the monitoring of pharmaceuticals in fish, whereas plasma is more inconvenient due to very low concentrations and collection difficulties. Elevated concentrations of certain metabolites suggest possible formation of human metabolites in fish. Measured concentrations indicate a low bioaccumulation potential for pharmaceuticals in fish tissues.

Zusammenfassung

Als Folge des Weltbevölkerungswachstums und des daraus resultierenden Wassermangels ist das Thema Wasserqualität zunehmend im Fokus der Öffentlichkeit. In diesem Kontext stellen anthropogene organische Stoffe —oft als Mikroschadstoffe bezeichnet— eine Bedrohung für die Wasserressourcen dar. Besonders Pharmazeutika werden aufgrund ihrer permanenten Einleitung, ihres steigenden Verbrauchs und ihrer wirkungsbasierten Strukturen mit besonderer Besorgnis diskutiert. Pharmazeutika werden hauptsächlich über Kläranlagen in die Umwelt eingeleitet, zusammen mit ihren Metaboliten und den vor Ort gebildeten Transformationsprodukten (TPs). Wenn sie die aquatische Umwelt erreichen, verteilen sie sich zwischen den verschiedenen Umweltkompartimenten, insbesondere der Wasserphase, Schwebstoffen (SPM) und Biota. In den letzten Jahrzehnten wurden Pharmazeutika in der Wasserphase umfassend untersucht. Allerdings wurden extrem polare Pharmazeutika aufgrund des Mangels an robusten Analysemethoden nur selten überwacht. Zudem wurden Metaboliten und TPs selten in Routineanalysemethoden einbezogen, obwohl ihre Umweltrelevanz nachgewiesen ist. Darüber hinaus wurden Pharmazeutika nur sporadisch in SPM und Biota untersucht und es fehlen adäquate Multi-Analyt-Methoden, um umfassende Ergebnisse über ihr Vorkommen in diesen Matrices zu erhalten. Die vorliegende Arbeit wird, diese Wissenslücken durch die Entwicklung generischer Multi-Analyt-Methoden zur Bestimmung von Pharmazeutika in der Wasserphase, SPM und Biota geschlossen und das Vorkommen und die Verteilung von Pharmazeutika in diesen Kompartimenten bewertet. Für einen vollständigen Überblick wurde ein besonderer Schwerpunkt auf polare Pharmazeutika, pharmazeutische Metaboliten und TPs gelegt. Insgesamt wurden drei innovative Multi-Analyt-Methoden entwickelt, deren Analyten ein breites Spektrum an physikalisch-chemischen Eigenschaften abdecken.

Zuerst wurde eine zuverlässige Multi-Analyt-Methode entwickelt um extrem polare Pharmazeutika, deren Metaboliten und TPs in wässrigen Umweltproben zu untersuchen. Die ausgewählten Analyten deckten einen signifikanten Bereich erhöhter Polarität ab und die Methode ist leicht um weitere Analyten erweiterbar. Diese Vielseitigkeit konnte durch die Verwendung der Gefriertrocknung als Probenvorbereitung und der zwitterionischen Hydrophile Interaktionschromatographie (HILIC) im Gradientenelutionsmodus erreicht werden. Die Eignung der HILIC-Chromatographie zur gleichzeitigen Quantifizierung einer großen Bandbreite von Mikroschadstoffe in wässrigen Umweltproben wurde gründlich untersucht. Es wurde auf mehrere Einschränkungen hingewiesen: eine sehr komplexe und zeitaufwändige Methodenentwicklung, eine sehr hohe Empfindlichkeit hinsichtlich der Änderung des Acetonitril-Wasser-Verhältnisses im Eluenten oder im Verdünnungsmittel und hohe positive Matrixeffekte für bestimmte Analyten. Diese Einschränkungen können jedoch durch die Verwendung eines präzisen Protokolls und entsprechend markierter interner Standards überwunden werden und werden durch die Vorteile von HILIC, die die chromatographische Trennung von extrem polaren Mikroverunreinigungen ermöglicht, überkompensiert. Die Untersuchung von Umweltproben zeigte erhöhte Konzentrationen der Analyten in der Wasserphase. Insbesondere Gabapentin, Metformin, Guanylharnstoff und Oxypurinol wurden bei Konzentrationen im µg/L-Bereich im Oberflächenwasser gemessen.

Für die Bestimmung von 57 Pharmazeutika und 47 Metaboliten und TPs, die an SPM sorbiert sind, wurde anschließend eine verlässliche Multi-Analyt-Methode etabliert, die eine Quantifizierung bis in den niedrigen ng/g-Bereich erlaubt. Diese Methode wurde konzipiert, um einen großen Polaritätsbereich abzudecken, insbesondere unter Einbeziehung extrem polarer Pharmazeutika. Das Extraktionsverfahren basierte auf einer Druckflüssigkeitsextraktion (PLE), gefolgt von einer Rei-

nigung durch Lösungsmittelaustausch und Detektion durch direkte Injektion-Umkehrphasen-LC-MS/MS und Gefriertrocknung-HILIC-MS/MS. Das Sorptionspotential der Pharmazeutika wurde anhand von Laborexperimenten untersucht. Abgeleitete Verteilungskoeffizienten K_d variierten um fünf Größenordnungen unter den Analyten und bestätigten ein hohes Sorptionspotential für positiv geladene und unpolare Pharmazeutika. Das Vorkommen von Pharmazeutika in SPM deutscher Flüsse wurde durch die Untersuchung jährlicher Mischproben bewertet, die zwischen 2005 und 2015 an vier Standorten am Rhein und einem Standort an der Saar entnommen wurden. Dabei zeigte sich das ubiquitäre Vorkommen von an SPM sorbierten Pharmazeutika in diesen Flüssen. Insbesondere positiv geladene Analyten, auch sehr polare und unpolare Pharmazeutika zeigten nennenswerte Konzentrationen. Für viele Pharmazeutika wurde eine deutliche Korrelation zwischen den jährlich in Deutschland konsumierten Mengen und den in SPM gemessenen Konzentrationen festgestellt. Untersuchungen der zusammengesetzten räumlichen Verteilung von SPM erlaubten es, durch den Vergleich der Verschmutzungsmuster entlang des Flusses Hinweise auf spezifische industrielle Einleitungen zu erhalten. Diese Ergebnisse zeigten zum ersten Mal das Potential von SPM für die Überwachung von positiv geladenen und unpolaren Pharmazeutika in Oberflächengewässern. Für Pharmazeutika mit erhöhter Sorptionsaffinität (K_d über 500 L/kg) erlauben SPM-Analysen sogar die Überwachung niedrigerer Emissionen.

Schließlich wurde eine zuverlässige und generische Multi-Analyt-Methode zur Untersuchung von 35 Pharmazeutika und 28 Metaboliten und TPs in Fischplasma, Fischleber und Fischfilet entwickelt. Für diese Matrix war es eine große Herausforderung eine adäquate Aufreinigung zu entwickeln, die eine ausreichende Trennung der störenden Matrix von den Analyten ermöglicht. Bei der endgültigen Methode wurde die Extraktion von Fischgewebe durch Zellaufschluss durchgeführt, gefolgt von einem nicht diskriminierenden Clean-up auf der Basis von Kieselgel-Festphasenextraktion (SPE) und Materialien mit eingeschränkter Zugänglichkeit (RAM). Die Anwendung der entwickelten Methode auf die Messung von Brassen- und Karpfengeweben aus deutschen Flüssen zeigte, dass selbst polare Mikroverunreinigungen wie z.B. Pharmazeutika in Fischgeweben ubiquitär vorhanden sind. Insgesamt wurden 17 Analyten zum ersten Mal in Fischgewebe nachgewiesen, darunter 10 Metaboliten/TPs. Die Bedeutung der Überwachung von Metaboliten und TPs in Fischgeweben wurde durch deren Nachweis in ähnlichen Konzentrationen wie bei ihren Ausgangsstoffe bestätigt. Es zeigte sich, dass Leber und Filet für die Überwachung von Pharmazeutika in Fischen geeignet sind, während Plasma aufgrund sehr niedriger Konzentrationen und erschwerter Probenahme ungeeignet ist. Erhöhte Konzentrationen bestimmter Metaboliten deuten auf eine mögliche Bildung menschlicher Metaboliten in Fischen hin. Die gemessenen Konzentrationen weisen jedoch auf ein geringes Bioakkumulationspotential für Pharmazeutika in Fischgeweben hin.

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General introduction

1.1 Water pollution

Humanity has been confronted with the problem of water pollution since its sedentism. Its first form was excrement pollution causing the spread of diseases, then from neolithic, heavy metal contamination [1]. These early contaminations were not without consequence on the development of human civilization and lead pollution is even believed by some authors to be responsible for Rome's decline [2].

Nevertheless, water pollution — and its consequence on the human being — took another extent with the beginning of the industrial era. Rural exodus caused a strong increase of the population density in urban centers and industry development was accompanied by the discharge of large amounts of wastes in the water bodies. This brought out the spread of epidemics with for instance the death of 3% of the English population due to cholera in 1848-9 [3] and impressive environmental disasters such as the 9-times inflammation of the Cuyahoga River between 1868 and 1969 due to oil spill [4].

Nowadays, water pollution takes many forms with various effects on humans and ecosystems.

- Pathogen contamination is one of its oldest and best controlled hazards. Still, waterborne pathogens cause the death of 2 million people each year, in particular related to unsuitable water treatment facilities in emerging countries [5].

- Heavy metals have high detrimental potential due to their elevated acute toxicity and bioaccumulativity. Their discharge occurs mostly via point source associated with industrial activities such as mining, foundries or smelters. In Minnamata (Japan), industrial discharge of methyl mercury caused the severe intoxication of 2000 people including 1000 deaths [6].

- Nutrients pollution or eutrophication is the over-enrichment of water bodies with minerals and nutrients inducing the excessive growth of algae. The decay of these supernumerary algae causes oxygen depletion and thus the death of aquatic organisms and the destruction of the corresponding ecosystem. This recent form of pollution takes its origins in the discharge of nitrogen and phosphate-containing fertilizers [7].

- Organic micropollutants gather anthropogenic compounds present at trace levels in the aquatic environment with potential danger for the aquatic organisms, ecosystem and human health. One of the most characteristic examples of micropollutants impact is Agent Orange pollution. This mixture of 2,4,5-trichlorophenoxyacetic acid,

2,4-dichlorophenoxyacetic acid with traces of 2,3,7,8-tetrachlorodibenzo-p-dioxin was largely spread over South Vietnam forests by the United States Army during the Vietnam War. Beyond the originally planned deforestation, long term damages were caused to the ecosystem due to the toxic effects of the dioxin [8]. Numerous adverse effects on human health were observed including increased cancer rates, reproductive and developmental abnormalities and nervous system disabilities [9].

Water pollution jeopardizes human health since prehistory. Some of its aspects, such as pathogen pollution are ancient and well understood. Other such as micropollutant pollution still need further investigations and are the source of great concerns due to their impact on ecosystems and human health.

1.2 Organic micropollutants

Organic micropollutants can be classified in persistent organic pollutants and contaminants of emerging concern. This classification is dynamic, contaminants of emerging concern may be reclassified as persistent organic pollutants after thorough investigations of relevant properties.

Persistent organic pollutants are anthropogenic substances combining persistence, mobility, bioaccumulativity and toxicity [10, 11]. Their resistance to environmental degradation and their semi-volatility caused their worldwide spread with particularly high concentrations in polar regions, where the low temperatures favor their condensation [10–13]. Persistent organic pollutants accumulate in the adipose tissues of living organisms due to their high lipophilicity [12] and are subject to food web magnification, i.e. their concentrations increase with trophic levels [14]. Persistent organic pollutants have a high toxicity potential due to their adverse effects on the endocrine system, reproduction and fetus development [10]. These detrimental effects were observed as well in humans as in aquatic and terrestrial biota. The main groups of persistent organic pollutants are polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), organochloride pesticides, polybrominated diphenyl ethers (PBDEs) and dioxins [10]. Persistent organic pollutants have been regulated as soon as the 1970s [15, 16]. The Stockholm convention has permitted the strong regulation of 12 persistent organic pollutants since 2004 and has been expanded to 16 supplementary substances during its revision in 2017 [17]. However, due to their stability, persistent organic pollutants are still regularly detected in sediments, aquatic organisms and human plasma [18, 19].

Contaminants of emerging concern gather anthropogenic micropollutants which have been recently detected or found to be significant and are suspected or known to have

adverse effects on human and their environment [20, 21]. These are compounds whose environmental impact is not fully understood yet and which are typically not regulated by environmental directives. Pharmaceuticals, personal care products, pesticides, illicit drugs, surfactants, organic dyes, sweeteners, plastic additives and some flame retardants are considered as contaminants of emerging concern. Among contaminants of emerging concern, pharmaceuticals are the source of major worries due to their effect designed structure.

1.3 Pharmaceuticals, a growing concern

Pharmaceuticals are defined as such by their curative effects and therefore cover a large range of biological activities and physico-chemical properties. Mostly, these are highly functionalized and polar molecules, since their bioavailability depends on their solubility [22, 23].

Pharmaceutical consumption has strongly increased throughout the 20^{th} century with the development of modern medicine and synthetic drugs. Currently, more than 1000 pharmaceutical active ingredients are referenced by the European Medicines Agency [24] and 20 to 60 new pharmaceutical active substances are commercialized each year [25]. In the last decade, in particular, pharmaceutical consumption soared. Illustratively, in OECD countries, antidiabetic drug consumption has increased by 100% between 2000 and 2015, cholesterol-lowering medications by 300%, antihypertensive drugs by 100% and antidepressants by 100% [26]. This can be attributed to the aging population, the prevalence of chronic diseases and changes in the clinical guidelines [26]. Due to the increase of the world population and its aging [27], further growth in pharmaceutical consumption in the next decades is inevitable, raising concern about the increasing introduction of pharmaceuticals in the environment.

1.4 Pharmaceutical pathways to rivers and streams

1.4.1 Sources

Pharmaceuticals are introduced into the environment via three main sources: industrial discharge, disposal of unused medications and their consumption (Figure 1.1).

Drug manufacture constitutes a non-negligible pathway of pharmaceuticals to the environment. This is particularly the case in China and India where a large part of the worldwide production is outsourced. In these countries, concentrations in the mg/L-g/L range have been measured in drug manufacture effluents [28]. Industrial discharges have

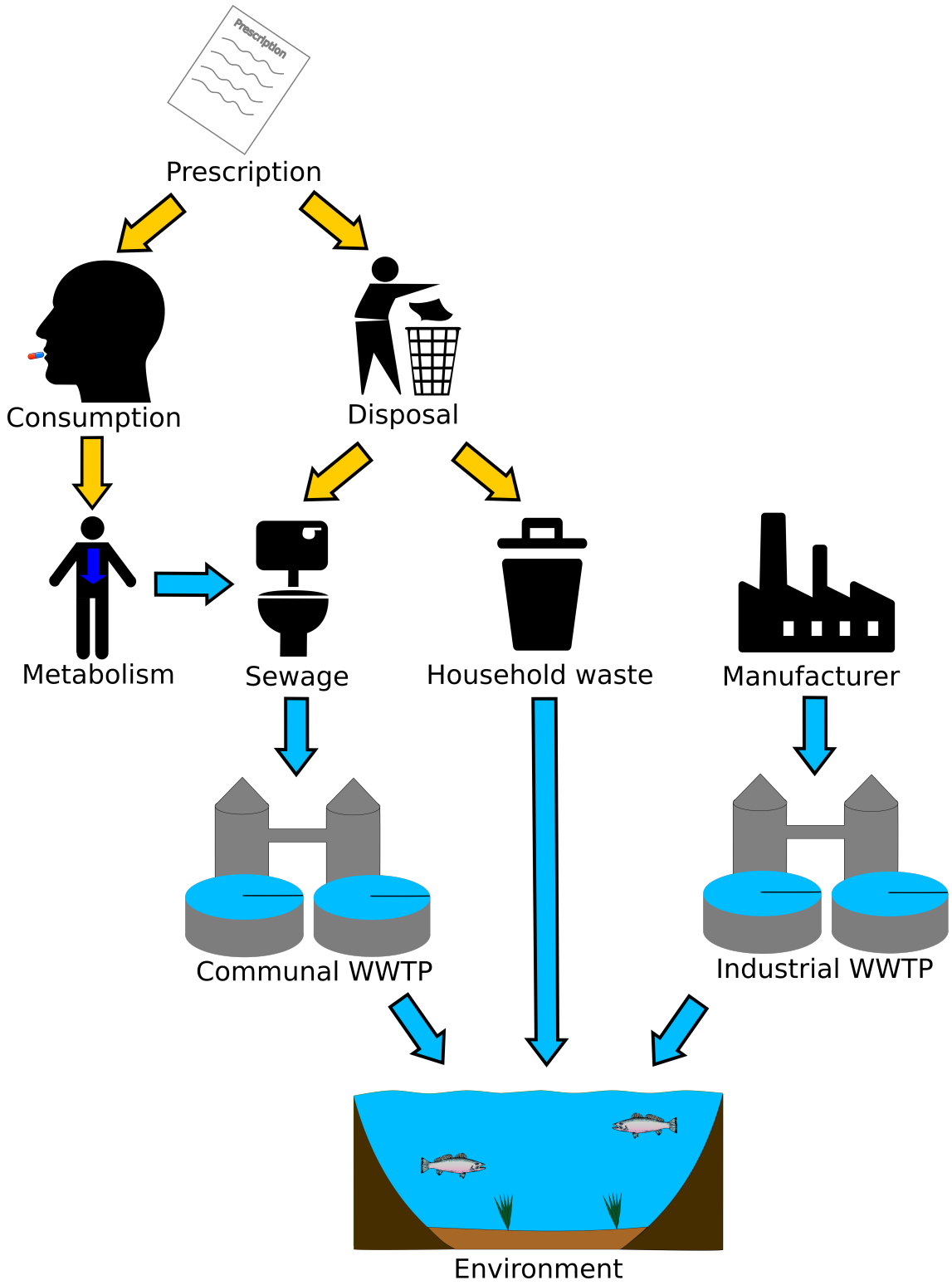

Figure 1.1: Pathways of pharmaceuticals to the environment

also been regularly reported for high-income countries with concentrations up to the low mg/L range [28, 29].

Non-adequate disposal of unused medication is an important introduction route to the environment. Patients tend to dispose of their pharmaceutical surplus in the garbage, the toilet or the sink instead of returning them to the pharmacy [30, 31]. This is a far from negligible phenomenon, as these practices have been reported by 72% of the patients in Ireland and 76% in the United Kingdom [30, 31]. Pharmaceuticals disposed of in sinks or toilets are conducted to the wastewater treatment plants (WWTPs) via sewage. Therefrom a certain proportion reaches the water bodies due to the inability of WWTPs to remove them comprehensively (see 1.4.3). Pharmaceuticals disposed of in the garbage are buried in landfill sites, where they are progressively leached into the environment [30].

Pharmaceutical consumption conducts ultimately to their discharge into the environment. Ingested pharmaceuticals are partially metabolized before excretion in urine and/or feces [32] (Table 1.1). Via toilet wastewater, the excreted parent pharmaceuticals and metabolites reach the WWTPs and, after passing the WWTPs, the water bodies. Consequently, it is primordial to apprehend drug metabolism to evaluate their environmental impact.

Table 1.1: Excretion data from literature

Name	Type	Excretion unchanged in urine [%]	Excretion unchanged in feces [%]	Total excretion [%]	Excretion as glucuronide [%]	Source
Abacavir	Parent	2	-[b]	2	23	[33]
Aciclovir	Parent	61-91	-[b]	76	n.a.	[34]
Aliskiren	Parent	0.4	77.5	77.9	n.a.	[35]
Amisulpride	Parent	23-46	20	55	n.a.	[36]
Atenolol	Parent	39	32	71	2	[37]
Hydroxyatenolol	Metabolite	3	<1	3	n.a.	[37]
Bezafibrate	Parent	52	-[b]	52	22	[38]
Bicalutamide	Parent	<1	31	31	19	[39]
Bisoprolol	Parent	50-60	-[b]	55	n.a.	[40]
Candesartan	Parent	-[b]	-[b]	75	<10-20	[41]
Carbamazepine	Parent	0.8	13	14	11	[42]
2-Hydroxycarbamazepine	Metabolite	4.3[a]	-[b]	4.3[a]	-[a]	[42]
3-Hydroxycarbamazepine	Metabolite	5.1[a]	-[b]	5.1[a]	-[a]	[42]
10-Hydroxy-10-hydroxycarbamazepine	Metabolite	<0.1	-[b]	<0.1	n.a.	[42]
10,11-Dihydroxy-10,11-dihydrocarbamazepine	Metabolite	32	-[b]	21	11	[42]
Cetirizine	Parent	60	10	70	n.a.	[43, 44]
Chlorothiazide	Parent	-[b]	-[b]	>50	n.a.	[45]
Citalopram	Parent	12-23	10	28	11	[46, 47]
Desmethylcitalopram	Metabolite	14	-[b]	14	n.a.	[46]
Didemethylcitalopram	Metabolite	7	-[b]	7	4.5	[46]
Clarithromycin	Parent	-[b]	-[b]	20	n.a.	[48, 49]
Clindamycin	Parent	10	-[b]	10	Reported	[50]
Clopidogrel	Parent	-[b]	-[b]	-[b]	n.a.	[51]
Clopidogrel carboxylic acid	Metabolite	-[b]	-[b]	-[b]	Reported	[52]
Diclofenac	Parent	1	15	16	4	[53–55]
4'-Hydroxy-diclofenac	Metabolite	0.7	15	40	11	[53, 54]
Diphenhydramine	Parent	0.1-4	6	8	8.8-13.2	[56]
Duloxetine	Parent	Not detected	0.1-4.1	2	n.a.	[57]

Name	Type	Excretion unchanged in urine [%]	Excretion unchanged in feces [%]	Total excretion [%]	Excretion as glucuronide [%]	Source
Emtricitabine	Parent	64	-[b]	64	4	[50]
Fexofenadine	Parent	11	80	85	n.a.	[58, 59]
Flecainide	Parent	10-50	-[b]	30	n.a.	[60]
Flecainide-meta-O-dealkylated	Metabolite	11-16[a]	-[b]	13[a]	-[a]	[60]
Fluconazole	Parent	80-90	-[b]	85	6.5	[61]
Fluoxetine	Parent	11	15	26	7	[53, 62]
Norfluoxetine	Metabolite	7	Not detected	7	8-20	[53, 62]
Furosemide	Parent	55	-[b]	55	11	[50, 63, 64]
Gabapentin	Parent	10-23	76-81	100	n.a.	[50]
Hydrochlorothiazide	Parent	83	18	100	n.a.	[53]
Irbesartan	Parent	4.3	30	34	6	[65]
Lamivudine	Parent	70	-[b]	70	n.a.	[66]
Lamotrigine	Parent	4.9-21	-[b]	10	56-63	[67, 68]
Levetiracetam	Parent	66	-[b]	66	n.a.	[69]
Levetiracetam acid	Metabolite	24	-[b]	24	n.a.	[69]
Lidocaine	Parent	10	-[b]	10	n.a.	[70]
Norlidocaine	Metabolite	4	-[b]	4	1.5	[70, 71]
Metamizole	Parent	Not detected	-[b]	Not detected	n.a.	[72]
4-Acetylaminoantipyrine	Metabolite	30	-[b]	30	n.a.	[72]
4-Formylaminoantipyrine	Metabolite	21	-[b]	21	n.a.	[72]
Metformin	Parent	50	27	77	n.a.	[50]
Metoprolol	Parent	6-10	-[b]	9	n.a.	[50, 73]
Hydroxy metoprolol	Metabolite	10	-[b]	10	n.a.	[74]
O-Desmethyl metoprolol	Metabolite	1	-[b]	1	n.a.	[74]
Metoprolol acid	Metabolite	65	-[b]	65	n.a.	[74]
Naproxen	Parent	10	-[b]	10	40	[75]
O-Desmethyl naproxen	Metabolite	5	-[b]	5	12	[75]
Olmesartan	Parent	10-16	65-90	80	n.a.	[76]
Oxazepam	Parent	Traces	10	10	70-80	[77, 78]

Name	Type	Excretion unchanged in urine [%]	Excretion unchanged in feces [%]	Total excretion [%]	Excretion as glucuronide [%]	Source
Phenytoin	Parent	0.2-0.7	0.7-20	6.1	n.a.	[50]
Pregabalin	Parent	90	n.a.	90	n.a.	[50]
Primidone	Parent	42	-[b]	42	n.a.	[50, 79]
Quetiapine	Parent	<1	<1	<1	n.a.	[80]
Methylphenidate	Parent	Not detected	-[b]	<1	n.a.	[50, 81]
Ritalinic acid	Metabolite	80	-[b]	80	n.a.	[81]
Roxithromycin	Parent	-[b]	-[b]	50	n.a.	[49]
Sertraline	Parent	<0.2	12-14	13	Reported	[50, 82]
Sildenafil	Parent	Not detected	Not detected	Not detected	n.a.	[83]
Sitagliptin	Parent	66	10	76	<1	[84]
Sotalol	Parent	80.1	12.5	93	n.a.	[85, 86]
Sulfamethoxazole	Parent	15	-[b]	15	8	[50, 87]
N-Acetyl sulfamethoxazole	Metabolite	46	-[b]	46	n.a.	[87]
Sulpiride	Parent	70	-[b]	70	n.a.	[50]
Tadalafil	Parent	<1	-[b]	<1	n.a.	[50]
Telmisartan	Parent	<1	98	98	n.a.	[88]
Torasemide	Parent	25	-[b]	25	n.a.	[89]
Hydroxytorasemide	Metabolite	12	-[b]	12	n.a.	[89]
Tramadol	Parent	10-30	-[b]	20	<2	[90–92]
Dehydrotramadol	Metabolite	<2	-[b]	<2	n.a.	[91]
O-Desmethyl tramadol	Metabolite	15	-[b]	15	2-5	[90, 91]
N,O-Didesmethyl tramadol	Metabolite	5-10	-[b]	20	2-5	[91]
Trimethoprim	Parent	46-47	-[b]	47	n.a.	[50, 93]
3-Desmethyl trimethoprim	Metabolite	1.1-3.8	-[b]	2.5	n.a.	[93]
Valsartan	Parent	10	71	81	n.a.	[94]
Valeryl-4-hydroxyvalsartan	Metabolite	1.1	8.0	9.1	n.a.	[94]
Venlafaxine	Parent	5	-[b]	5	n.a.	[95]
N-Desmethyl venlafaxine	Metabolite	1	-[b]	1	n.a.	[95]
O-Desmethyl venlafaxine	Metabolite	29	-[b]	29	26	[95]

Name	Type	Excretion unchanged in urine [%]	Excretion unchanged in feces [%]	Total excretion [%]	Excretion as glucuronide [%]	Source
N,O-Desmethyl venlafaxine	Metabolite	10	-[b]	10	6	[95]
Xipamide	Parent	40	-[b]	40	33	[96]

[a] Conjugate included

[b] To the best of our knowledge, no published data

1.4.2 Metabolism

Due to the presence of poisonous molecules in plants (phytoalexin), most species have developed defense mechanisms to detoxify exogenous molecules and facilitate their excretion. This mechanism —the metabolism— proceeds by enzyme cascade and has a broad substrate specificity due to the structure variety of phytoalexins. Therefore, it acts on a large range of synthetic chemicals including pharmaceuticals. It is typically divided into phase I and phase II metabolism, whereby most pharmaceuticals undergo them sequentially [22, 97].

Phase I reactions are functionalization reactions, i.e. functional groups are formed or already existing functional groups are set free. They include mainly oxidation, reduction, hydrolysis, hydration and isomerization. If phase I metabolites are polar enough they may be excreted at this point, otherwise they commonly undergo phase II metabolism [97, 98].

Phase II reactions are conjugation reactions, functional groups are conjoined with negatively charged endogenous molecules. Main phase II reactions are glucuronidation, sulfation, methylation, acetylation and conjugation with amino acids or glutathion [97, 98].

1.4.3 Fate of pharmaceuticals in WWTP

In the vast majority of cases, pharmaceuticals are discharged in the environment via communal or industrial WWTP. However, conventional WWTPs are not designed to eliminate organic micropollutants but to reduce the organic matter and nutrients loads (nitrogen, phosphate). In consequence, pharmaceuticals are only partly removed in the wastewater treatment and elimination rates vary strongly according to their physico-chemical properties and bioavailability [99] (Table 1.2).

Conventional WWTPs include three main steps: pre-treatment, primary treatment and secondary treatment.

Pre-treatment permits the elimination of solid residues contained in the sewage. It is a mechanical step proceeding via coarse screening and grits. The resulting sewage is placed in a sedimentation tank over several hours for the primary treatment. After this time-lapse, suspended particles matter have settled by gravity and the supernatant can be transferred to the secondary treatment. Secondary treatment aims at removing the organic matter and excess nutrients from the sewage. Mostly, the activated sludge process is utilized. For this purpose, the primary processed effluent is placed in a series of tanks containing a high amount of micro-organisms. Anaerobic and aerobic conditions are alternated in a cyclic process, allowing the extensive degradation of organic matter via microbial reactions. After several hours, the effluent is transferred in a secondary clarifier

to separate the sludge from the water phase. Subsequently, the supernatant is discharged in the water bodies or undergo supplementary advanced treatments such as membrane process, ozonation or granulated activated carbon filtration [31, 100].

In a conventional WWTP, the fate of pharmaceuticals is controlled by two main processes: sorption and biodegradation [100].

Sorption occurs throughout the WWTP treatment, on sludge, suspended particulate matter (SPM), sand or coarse debris [100]. As stated in 1.5.1.1, it primarily affects apolar and/or positively charged pharmaceuticals. However, it is not a removal process since sorbed pharmaceuticals are likely to desorb later, especially when sludge is used as fertilizer in the landfills [100].

Biodegradation during the secondary treatment is a largely uncontrolled process strongly dependent on the nature and structure of the microbial communities [101]. These ones are strongly affected by the operating conditions of the WWTPs in particular, pH, temperature, redox potential and dioxygen content as well as by the composition of wastewater [100, 102]. They have notably the ability to adapt to the present pharmaceuticals to degrade them more efficiently. In consequence, the biodegradation process has a high variability. In the ideal case, biodegradation leads to the mineralization of pharmaceuticals, i.e. their degradation in carbon dioxide, water and nitrate. However, for a lot of pharmaceuticals, this step is not achieved and the process stopped after the formation of one or several stable transformation products (TPs) [103]. Illustratively, acyclovir is removed at 97% in WWTP but is transformed thereby quantitatively into the stable carboxy-acyclovir which is discharged in the aquatic environment [104, 105]. Moreover, some pharmaceuticals are resistant to biodegradation such as the antiepileptic carbamazepine which is barely removed in conventional WWTPs [106].

Abiotic degradation (photolysis, hydrolysis, oxidation) or volatilization may also occur in WWTPs but have only a limited influence on pharmaceutical fate [102, 107].

Table 1.2: WWTP removal values reported in the literature

Name	WWTP removal [%]
4-Acetylaminoantipyrine	55 [108]
Abacavir	>99 [104], >99 [109]
Aciclovir	97-98 [104], 91 [109]
Aliskiren	25 [110]
Amisulpride	No significant removal [111]
Atenolol	<0-85.1 [112], 78 [113], 15-30 [114], 36 [115], 41 [116], 77 [117], 20-97 [118]
Atenolol acid	Formed [119]
Bezafibrate	40 [120], 50-70 [121], 9.1-70.5 [112], 45-55 [114], 23-99 [118]
Bicalutamide	10-20 [122], 3 [123]
Bisoprolol	10-30 [114]
Candesartan	No significant removal [114]
Carbamazepine	3-10 [124], <0-62.3 [112], -22-10 [114], -12 [125], 28 [117], -5 [126]
Cetirizine	No removal [127], 16 [128], no removal [129]
Chlorothiazide	-30 [122]
Citalopram	33-36 [130], -2-15 [114], 18 [125], 29 [131]
Desmethylcitalopram	41-42 [130]
Clarithromycin	40 [125], -34 [117], -45-54 [131]
Clindamycin	-75- -15 [114], 55 [126]
Clopidogrel	35 [126]
Clopidogrel carboxylic acid	-80 [126]
Diclofenac	-10-40 [124], 20-30 [132], <0-81.4 [112], 30-100 [118]
Diphenhydramine	94 [117], 69 [133], 60 [126]
Emtricitabine	74 [109]
Fexofenadine	11 [125]
Fluconazole	5-25 [114], 13 [134]
Fluoxetine	70 [108], 65 [130], 35 [117]
Norfluoxetine	90 [130]
Furosemide	35-75 [121], 30 [122], 97 [117], 20-96 [118], 8 (sommer) [131], 54 (winter) [131]
Gabapentin	-2-18 [114], >99 [131]
Hydrochlorothiazide	23-31 [124], 5 [117], 24-76.3 [131]
Irbesartan	-42-6 [124], -2-18 [114]
Lamivudine	>76->93 [104]
Lamotrigine	-30–70 [114]
Levetiracetam	98 [114]
Lidocaine	30-50 [70]
Metformin	98-99 [124], 99 [117]
Metoprolol	29-42 [124], 3-56.4 [112], 29 [113], -20-2 [114], 36 [117]
Naproxen	90 [120], 60-80 [121], 86.2-95.8 [116], 100 [117], 60-100 [118]
Olmesartan	7-21 (lab-scale sewage plants) [135]
Oxazepam	-38-26 [136], -17 [125]
Phenytoin	44 [131]
Pregabalin	50-60 [114]
Primidone	-5-20 [114], <-5 [131]
Quetiapine	>67 [114], 60 [126]
Ritalinic acid	23 [137]
Roxithromycin	-80-61 [131]
Sertraline	81 [125], 82 [130], 11 [131], 50 [126]
N-Desmethyl sertraline	35 [130], -150 [138]

Name	WWTP removal [%]
Sertraline ketone	52 [130]
Sildenafil	68 [139]
Sotalol	17-24 [124], 25 [113], -1-15 [114]
Sulfamethoxazole	70 [121], 4-88.9 [112], 10-70 [114], 21-99.5 [116], 58 [125], 66 [117], 30-92 [118], 35 [126]
Sulpiride	0-30 [111]
Tadalafil	69 [139]
Telmisartan	20-70 [114]
Torasemide	10-30 [114]
Tramadol	30 [121], -5-5 [114], 40-50 [136]
O-Desmethyl tramadol	0 [136]
Trimethoprim	40-60 [121], <0-81.6 [112], -22-1 [114], 21.2-98 [116], 20 [125], -2 [117], 55 [126]
Valsartan	89-95 [124], 40-75 [121], 43.3-98.6, 15-35 [114]
Venlafaxine	50 [120], 0-15 [114], 1 [125], 18 [130], 35 [126]
O-Desmethyl venlafaxine	No significant removal [130]
N,O-Desmethyl venlafaxine	No significant removal [130]

1.5 Fate of pharmaceuticals in the environment

1.5.1 Distribution

Once entered in the water bodies, pharmaceuticals distribute between the different environmental compartments: water, air, SPM, sediment and biota (Figure 1.2). Water/air distribution is controlled by volatility, this mechanism will not be detailed since it is of minor extent for pharmaceuticals. Water/SPM and water/sediment partitions are controlled by sorption (see 1.5.1.1) whereas water/biota partition is governed by bioaccumulation (see 1.5.1.2). This distribution plays a fundamental role in the transport of pharmaceuticals worldwide, since the flow dynamics of water, SPM, sediment and biota differ widely [103].

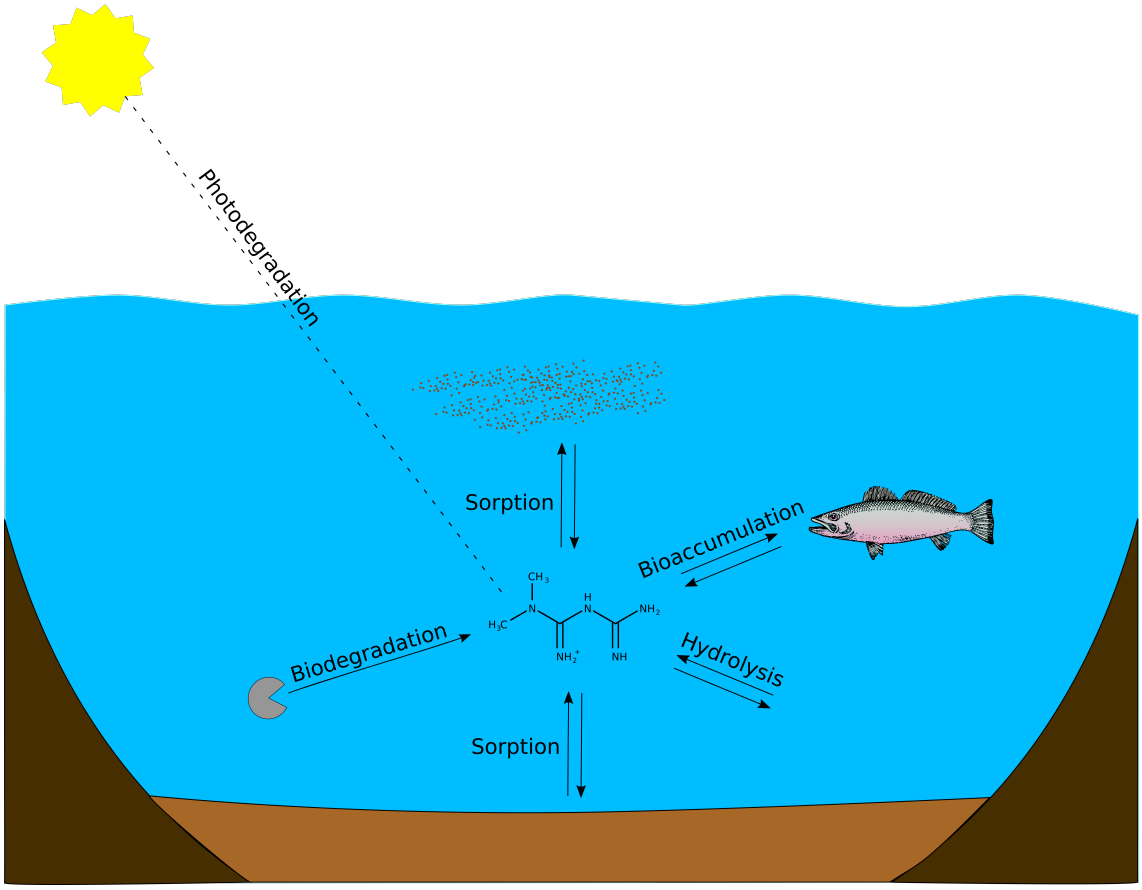

Figure 1.2: Fate of pharmaceuticals in the aquatic environment

1.5.1.1 Sorption

Sorption is mainly controlled by two mechanisms: hydrophobic partitioning and electrostatic interactions. Hydrophobic partitioning corresponds to the distribution of the phar-

maceuticals between the polar water phase and the nonpolar organic matter of sediments or SPM. This process is controlled by the solvation energy of pharmaceuticals and their Van der Waals interactions with the organic matter surface. It favors thus the sorption of pharmaceuticals containing a hydrophobic moiety. Electrostatic interactions include ion exchange and reversible complexation reactions. On SPM and sediment surface, there is a strong predominance of negatively charged sites, since as well the organic matter surface (via carboxylate and hydroxyl groups) and the clay minerals (via aluminosilicates) are globally negatively charged at surface water pH. In consequence, ion exchanges are mainly cationic favoring the sorption of pharmaceuticals containing a positively charged moiety. Complexation reactions are rarer and involve mostly anionic sorbents either via cations bridging or surface complexation reactions. For most pharmaceuticals, both electrostatic interactions and hydrophobic partitioning are involved, making the global sorptive interactions extremely complex to evaluate [140].

The Freundlich equation is commonly utilized to describe pharmaceutical sorption to sediment or SPM. It is based on an empirical model which assumes that the involved surface is heterogeneous and the sorbed amount increases indefinitely with increasing concentrations [141–143]. For sorption processes involving partition between the water phase and a solid, it is defined by the equation 1.1:

$$C_s = K_f C_w^{1/n} \tag{1.1}$$

with:

C_s = concentration of the pharmaceutical in the solid phase

C_w = concentration of the pharmaceutical in the water phase

K_f = Freundlich constant, measurement of the sorption capacity

n = Freundlich exponent, measurement of the variation of free energy associated with sorbent sorption

In the particular case where the free energy varies little, Freundlich exponent is close to 1 and the simplified linear model can be applied (equation 1.2):

$$C_s = K_d C_w \tag{1.2}$$

with:

K_d = partition coefficient

When the assumptions of the Freundlich model are not fulfilled — e.g. when the

number of sorption sites is limited—, the Langmuir model is commonly used to describe micropollutants sorption to sediment or SPM [142]. This model is based on the hypothesis that i) all sorption sites are equivalent in energy and localized, ii) the absorbed molecules do not interact with one other and iii) there is only monolayer absorption [141]. For sorption involving partition between the water phase and a solid, it can be expressed by the following equation:

$$C_s = \frac{Q_0 b C_w}{1 + b C_w} \tag{1.3}$$

with:

Q_0: concentration of the pharmaceutical in the solid phase when all sorption sites are occupied

b: adsorption coefficient

1.5.1.2 Bioaccumulation

Bioaccumulation corresponds to the concentration of a xenobiotic in an organism relative to the ambient environmental medium. It can be derived by the difference between the uptake processes — respiratory, dermal and dietary absorption — and the elimination processes — respiratory exchange, metabolism, excretion and growth dilution [144].

For aquatic organisms, respiratory absorption at the gills is generally the main uptake mechanism [145]. This complex process is mainly controlled by the diffusion of the pharmaceutical through the different layers of the gill epithelium up to blood vessels. Due to the low permeability of cell membranes to ions, the absorption of neutral compounds is favored [146]. However, respiratory absorption of ionized xenobiotics has also been reported and is attributed to the following mechanisms: i) ions act as a reserve for their neutral form and permit thus to maintain a high diffusion flux, ii) cell membranes are not totally impermeable to ions which can be transported via the epithelial cell junction or in association with counter-ions, iii) pH is lower at gill surface than in the bulk water due to the release of carbon dioxide, supporting the diffusion of weak acids (and disfavoring that of weak bases) [146, 147].

Dermal absorption is governed by similar mechanisms as respiratory absorption. For most aquatic organisms, it plays less of a role due to the thickness of the skin and its limited surface area and blood perfusion compared to the gills [148].

Dietary ingestion corresponds to the oral absorption of pharmaceuticals either directly from water or from contaminated food or particles. Ingested pharmaceuticals are uptaken via the gastrointestinal epithelium. This pathway permits the trophic transfer of pharmaceuticals and thereby the biomagnification of micropollutants with low biotransformation

and excretion potential [145].

Similarly to humans, aquatic organisms can partly metabolize pharmaceuticals. However, their substrate specificity is more narrow since they possess less cytochrome P450 forms than mammals [147].

1.5.2 Attenuation

1.5.2.1 Biodegradation in surface water

In rivers, the flowing water offers only low biodegradation ability. In consequence, biodegradation occurs principally on biofilms, layers of microorganisms adhering to solid surfaces located on bed sediments, SPM or submerged macrophytes [149, 150]. The effectiveness of biodegradation depends on the contact area between pharmaceuticals and biofilms and especially on the exchanges between the bulk water and the hyporheic zone. When these are high, elevated biodegradation rates can be achieved [151, 152]. Yet, high contact areas require shallow rivers, high flow rates and permeable sediment beds and are thus rather the exception than the rule. For surface water not falling into this category, biodegradation is only relevant for substances with high biodegradability such as ibuprofen [149, 153].

1.5.2.2 Photodegradation in surface water

In surface water, pharmaceuticals are exposed both to direct and indirect photolysis. Direct photolysis corresponds to the absorption of sunlight by the pharmaceuticals themselves, causing bond cleavage or molecular re-arrangement. It affects only certain pharmaceuticals since it requires a spectrum overlap between sunlight emission (λ>290 nm) and pharmaceutical absorbance. Indirect photolysis occurs when high reactive species —e.g. singlet oxygen or hydroxyl radicals— formed via the absorption of sunlight by nitrate or organic matter react with organic micropollutants. This process is less specific than direct photolysis [154–156].

Most pharmaceuticals are reactive toward indirect or direct photodegradation to some extent [157]. However, the significance of photodegradation as an environmental dissipation mechanism is difficult to evaluate from laboratory studies. A few studies performed in environmental conditions permit a better understanding. In an investigation performed over the 12 km stretch of the shallow Santa Ana River (0.3 m, California, USA), Lin et al. attribute removal rates of 87-91% for naproxen, 14% for gemfibrozil and 12% for ibuprofen to photodegradation [158]. Kunkel et al. show that photodegradation is a relevant elimination mechanism for diclofenac in shallow rivers (15 cm depth) whereas this is not the case in deeper rivers (1 m) [151]. This can easily be imputed to water absorbency filtering

sunlight since the majority of UV radiation is estimated to be absorbed within 2 m depth [150]. Consequently, photodegradation is a relevant degradation mechanism in shallow rivers, wetlands and lakes, but even there, significant dissipation rates were mostly determined for highly photolabile pharmaceuticals (e.g. diclofenac, naproxen) [151, 158–160]. In large rivers, photodegradation is probably mostly insignificant. Moreover, photodegradation seldom leads to mineralization and for several drugs, photodegradation TPs turn out to be more toxic than their parents [154, 156].

1.5.2.3 Hydrolysis

Whereas certain micropollutants can be significantly removed via hydrolysis, this degradation mechanism is negligible for most pharmaceuticals. This can be largely attributed to the conception of pharmaceuticals for oral intake [153, 161]. However, this degradation mechanism could play a significant role in the degradation of beta-lactam antibiotics in surface water [162, 163].

To conclude, several dissipation and distribution mechanisms participate in the attenuation of pharmaceutical concentrations in the water bodies. However, dissipation processes are substance-specific and require adequate environmental conditions to be effective. Moreover, they rarely lead to the mineralization of the pharmaceuticals, but to the formation of TPs with variable toxicity. Distribution mechanisms decrease pharmaceutical concentrations in the water phase but can favor their mobility and adverse effects on the ecosystems.

1.6 Hazards of pharmaceuticals in the environment

1.6.1 Risks for ecosystems

Due to their effect-based structures and their permanent discharge, the presence of pharmaceuticals in the environment is the object of major concern for the ecosystems. Aquatic organisms are particularly exposed since they are in permanent contact with the pollutants rejected in the water bodies. Pharmaceutical adverse effects are complex to estimate since metabolic pathways differ according to species. Thus, the same pharmaceutical can be harmless for one species and extremely toxic for another.

The introduction of pharmaceuticals in the environment has already caused major damages to some ecosystems. For instance, the use of diclofenac in livestock provoked the spectacular decline of five vultures species (-95% in 10 years) in the Indian Subcontinent. This was attributed to renal failures induced even at very low diclofenac concentrations in

these species [164]. The implications for the ecosystem were severe since vultures usually eliminated cattle carcasses reducing the spread of diseases. 17-α-ethynylestradiol was also shown to be highly damageable to the ecosystems. This synthetic hormone causes feminization and altered sex ratio in fish species at the concentrations present in WWTP effluent or surface water with high wastewater proportion [165, 166]. In Canada, a study at a lake scale showed that permanent exposure to this range of concentrations causes the depletion of the fathead minnow population within two years [167].

Other pharmaceuticals provoke more subtle detrimental effects. This is especially the case for active ingredients influencing foraging, predator avoidance and mate attraction, such as the antidepressants SSRIs (selective serotonin reuptake inhibitors) and SNRIs (serotonin-norepinephrine reuptake inhibitors) [168]. Illustratively, fluvoxamine induces spawning in mussels at concentrations as low as 0.03 µg/L [169] and fluoxetine influences the antipredator behavior of mosquito fish at low ng/L concentrations (e.g. in the range of environmental concentrations) [170]. Such behavior shifts endanger the renewal of the concerned species and can thus strongly destabilize the ecosystems over the long term.

Moreover, in the water bodies, the aquatic organisms are not exposed to one but a multitude of pharmaceuticals. It is still poorly understood how the simultaneous exposure to several pharmaceutical active ingredients affects an organism, in particular when their modes of action differ. However, it has been shown that the toxicity is often additive or even synergic and that a mixture can have considerable adverse effects even if all components are present in concentrations inferior to their toxicity thresholds [103, 171]. Thus, due to their extent and ubiquity, these so-called cocktail effects represent probably the main threat to the ecosystems.

Furthermore, pharmaceutical metabolites and TPs can also exert adverse effects on wildlife. Frequently, metabolites concentrations in the water bodies are superior to those of their parents whereas their toxicity can be similar. Some parent pharmaceuticals are degraded to a large extent in WWTPs or surface water and formed thereby stable TPs whose toxicity is highly variable but can exceed that of their precursor [172, 173]. Thus, as well metabolites as TPs may contribute to a significant extent to the cocktail effects.

To summarize, the global impact of pharmaceuticals in the environment on the ecosystems is extremely difficult to evaluate but is probably substantial.

1.6.2 Risks for humans

The presence of pharmaceuticals in the environment raises concerns about their consequences on human health, too.

Drinking water constitutes an important potential exposure pathway to pharmaceu-

ticals. Actually, in high-income countries, several active ingredients were measured at concentrations in the pg/L to low ng/L range in tap water [174–177]. Moreover, certain pharmaceuticals — in particular antibiotics— were reported at concentrations up to the high ng/L range in Chinese tap water [178] and up to the µg/L range in well water destined to human consumption in India [179].

Exposition to pharmaceuticals also occurs through nutrition. In meat and fish from intensive farming, pharmaceutical concentrations in the µg/g range were reported in high-income countries [180, 181] and concentrations in the mg/g range were even reached in meat from developing countries [182]. Vegetables and fruits can also show appreciable concentrations due to irrigation with contaminated water or fertilization with biosolids issued from WWTPs [183–185].

Thus, exposure to pharmaceuticals via drinking water and food has been confirmed. It is generally considered to represent a *de minimis* risk for human health [174–177, 186]. However, it is less obvious, if one considered that i) most studies examine only one source of exposure and thus underestimate the effective exposition, ii) risk factors are mostly calculated for average adults, whereas toxicological relevant levels are lower for fetuses, infants, pregnant women and allergic people, iii) risk studies focus mostly on high-income countries where the water quality is better controlled than in developing countries [183].

Moreover, detrimental effects on ecosystems can also affect human health. For instance, the diminution of the vulture population in India (see 1.6.1) causes an increase of the feral dog population. In consequence, human rabies incidences increase resulting in 45000 additional deaths in this area [183].

More specifically, the presence of antibiotics in the environment contributes to the spread of antibiotic-resistant bacteria populations. As a result, the treatment of well-known infectious diseases can become challenging and even lead to death. It is estimated that in 2014, 700 000 people died worldwide owing to antimicrobial resistance and current estimations predict 10 million antimicrobial resistance-related deaths every year from 2050 if no measure is taken [187, 188].

1.7 Occurrence of pharmaceuticals in the environment: state of knowledge

Although the first detection of pharmaceuticals in the aquatic environment dated from the 1970s with the measurement of clofibric acid in WWTP effluent [189], it is only in the 1990s-2000s that pharmaceutical environmental analysis generalizes with the improvement of chromatographic and detection performances in GC-MS(/MS) and later in LC-MS/MS

[190].

Thirty years later, the ubiquity of pharmaceuticals in the environment has been established [103, 191]. A comprehensive literature review conducted by Beek et al. shows that altogether 571 parent pharmaceuticals have been investigated in the environment with 501 showing positive detection(s) [191]. Additionally, 142 pharmaceutical metabolites and TPs have been analyzed with 127 of them detected at least once. Pharmaceuticals have been investigated in 71 countries all over the world, even if measurements in high-income countries were strongly overrepresented [191].

Surface water is the most investigated matrix with more than 45000 published environmental concentrations and 15000 positive detections. Measured concentrations range mostly from low to high ng/L levels and the median over positive detections is only 30 ng/L. The most investigated pharmaceutical classes are analgesics, antibiotics, beta-blockers, estrogens and lipid regulators. The highest concentrations were measured for antibiotics in surface water from India or China and reach the mg/L range. In total, 345 parent pharmaceuticals and 113 metabolites/TPs have been investigated in this matrix. However, only 974 published environmental concentrations correspond to metabolites/TPs overall. Some analytes were widely analyzed such as diclofenac (3280 published environmental concentrations) or carbamazepine (2513), whereas others were underrepresented such as metformin [191]. Indeed, only 36 environmental concentrations were reported for metformin [191], whereas it is the most prescribed pharmaceutical worldwide [192]. This underrepresentation of extreme polar pharmaceuticals, metabolites and TPs could be observed for all matrices and is related to the difficulties to develop adequate analytical methods.

For groundwater, more than 3000 published environmental concentrations were reported corresponding to 1269 positive detections mostly in the ng/L range (median: 29 ng/L). 119 parent pharmaceuticals were investigated and 21 metabolites/TPs with only 80 measurements for metabolites/TPs. Analgesics, antibiotics, beta-blockers, estrogens and lipid regulators were the most investigated classes with analgesics and lipid regulators showing the highest concentrations. Concentrations in the µg/L range were occasionally detected in landfill sites and in large cities where there is a higher risk for infiltration of wastewater to groundwater [191, 193].

For sediment, only 2000 published environmental concentrations were reported overall including 865 positive detections. They correspond to the investigation of 83 parent pharmaceuticals and 29 metabolites/TPs. Concentrations levels lie mostly in the low ng/g range and the median measured concentration is 7 ng/g. For this matrix, there has been a strong focus on antibiotics which represent almost half of the measurements. Antibi-

otics also show the highest measured concentration levels up to 1000 ng/g. For SPM, less than 400 environmental concentrations have been published including only 74 positive detections. They correspond to 29 parent pharmaceuticals and one metabolite/TP. Most measured concentrations were in the ng/g range and the median concentration was 34 ng/g. Analgesics and antidepressants were the most detected pharmaceutical classes in this matrix and showed the highest concentrations reaching the high ng/g range [191].

According to Miller et al., 1001 measured concentrations were published in fish monitoring studies corresponding to 490 positive detections [194]. In total, 111 parent pharmaceuticals were investigated and 7 metabolites/TPs. Concentrations lie mostly in the low ng/g range for tissues and in the low ng/ml range for plasma. Antibiotics and antidepressants were the most investigated classes. In plasma, the highest concentrations have been determined for antibiotics and NSAIDs (nonsteroidal anti-inflammatory drugs), whereas in tissues similar concentrations have been determined for the different pharmaceutical classes [194].

The occurrence of pharmaceuticals in the environment was broadly investigated in the last decades in particular in the water phase. However, there is still a gap of knowledge concerning the occurrence of pharmaceutical metabolites and TPs which have been hardly considered until recently. Moreover, polar pharmaceuticals have often been overlooked due to the lack of appropriate analytical methods. SPM and biota compartments suffer from a lack of consideration especially concerning the analysis of metabolites and TPs.

1.8 Objectives

The objective of this thesis was to cover the gaps of knowledge concerning the occurrence of pharmaceuticals in the aqueous environmental matrices, via the investigation of extreme polar pharmaceuticals, pharmaceutical metabolites and TPs in the water phase and the determination of pharmaceuticals, pharmaceutical metabolites and TPs in SPM and fish tissues. Moreover, it was aimed to evaluate the optimal strategy for the determination of pharmaceuticals with various physico-chemical properties in the different environmental compartments.

For that purpose, 61 pharmaceuticals were selected for their environmental relevance but also to cover a broad range of physico-chemical properties. Their corresponding environmental relevant metabolites and TPs were also examined and the inclusion of relevant polar analytes was particularly considered.

The first aspect of this work was the development of a multi-residue method for the determination of polar pharmaceuticals in the water phase **(Chapter 2)**. Polar compounds are often neglected due to the lack of adequate chromatographic methods. For their investigation, we selected hydrophilic interaction liquid chromatography (HILIC) for its complementary with commonly used reversed-phase column concerning the polarity domain of retention. The challenge was to develop a robust method to analyze pharmaceuticals with a broad structural and functional diversity down to the low ng/L range and applicable to complex matrices such as treated wastewater. The relevance of HILIC concerning the analysis of polar pharmaceuticals was thoroughly examined via a detailed inspection of matrix effects and robustness of the chromatographic separation.

Subsequently, an analytical multi-residue method was developed for the determination of pharmaceuticals in SPM and was applied to the determination of pharmaceutical time trends in Rhine and Saar SPM between 2005 and 2015 as well as to the evaluation of pharmaceutical sorption ability to SPM **(Chapter 3)**. The challenge was to develop a generic extraction and a generic clean-up applicable to the broad polarity range of the analytes and still attain limits of quantification in the low ng/g range. The relevance of SPM to testify of the environmental burden of pharmaceuticals was evaluated and the correlation of the concentrations with German pharmaceutical consumption was studied.

The third investigated environmental compartment was biota, which was studied through

the representative fish species. For this purpose, a multi-residue method was developed to determine the selected pharmaceuticals in fish fillet, liver and plasma. This method was applied to the analysis of fish tissues and plasma from German surface water with different wastewater proportions **(Chapter 4)**. For this matrix, it was especially challenging to develop an adequate clean-up allowing for the sufficient separation of the matrix disturbances from the analytes. The applicability of fish analysis to estimate the pharmaceutical burden in the environment was evaluated as well as the relevance of metabolites in the framework of fish analysis.

1.9 Outline

Development and application of a multi-residue method for the determination of polar pharmaceuticals in the water phase

Chapter 2 describes the development and validation of an analytical method for the determination of extreme polar pharmaceuticals in the aquatic environment with a particular accent on the evaluation of chromatographic robustness and matrix effects. The developed method was applied to examine the occurrence of polar pharmaceuticals in drinking water, groundwater, surface water and WWTP effluent.

Development and application of a multi-residue method for the determination of pharmaceuticals in SPM

Chapter 3 describes the development and validation of an analytical method for the measurement of pharmaceuticals, pharmaceutical metabolites and TPs in SPM. This method was designed for the determination of partition coefficients to SPM as well as to investigate the occurrence of pharmaceuticals in annual composite SPM samples from four sites at the Rhine (Weil, Iffezheim, Koblenz, Bimmen) and one site at the Saar (Rehlingen) between the years 2005 and 2015.

Development and application of a multi-residue method for the determination of pharmaceuticals in fish tissue

Chapter 4 describes the development and validation of an analytical method for the analysis of pharmaceuticals, pharmaceutical metabolites and TPs in fish fillet, liver and plasma. This method was applied to determine the occurrence of pharmaceuticals in fish taken from rivers and a canal with different wastewater proportions as well as monitoring ponds fed by effluents from municipal WWTPs.

Final conclusions

Chapter 5 summarizes the conclusions from these studies and their corresponding outcomes.

2

Utilization of large volume zwitterionic hydrophilic interaction liquid chromatography for the analysis of polar pharmaceuticals in aqueous environmental samples: benefits and limitations

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Abstract

Benefits and limitations of HILIC were studied for the analysis of extreme polar organic contaminants in aqueous environmental matrices. A sensitive analytical method was developed and validated for the detection of 11 pharmaceuticals, 15 pharmaceutical metabolites and transformation products and the artificial sweetener acesulfame in aqueous environmental samples. The analytical method consisted of a simple and non-specific sample preparation based on freeze-drying followed by detection with large injection volume (70 µL) zwitterionic HILIC-ESI-MS/MS. Robustness studies showed a high sensitivity of the retention times and peak shapes to variations of the acetonitrile/water ratio of both the eluent and the diluent. Thus, a thorough sample and eluent preparation is required to obtain reproducible results. Extreme matrix effects of >200% were observed for emtricitabine and acyclovir, which could be traced to the co-elution of nitrate and chloride, respectively. These matrix effects and those of other analytes could be efficiently compensated by using deuterated, ^{13}C and ^{15}N-labeled internal standards. The developed method was able to detect the selected 27 analytes in treated wastewater, surface water and groundwater down to limit of quantification (LOQ) in the lower ng/L range. Appreciable concentrations were detected, ranging up to 110 µg/L (guanylurea) in treated wastewater, up to 5.1 µg/L (oxipurinol) in surface water and up to 6.1 µg/L (acesulfame) in groundwater. In a German drinking water, only the X-ray contrast medium diatrizoate and the artificial sweetener acesulfame were quantified above LOQ with 0.19 µg/L and 0.35 µg/L, respectively.

2.1 Introduction

For decades, pharmaceuticals and their metabolites have been released into the aquatic environment via the discharge of municipal and industrial wastewater treatment plants (WWTP) or even terrestrial or agricultural run-off [103, 190, 195]. Pharmaceuticals are metabolized in humans and are further transformed in biological wastewater treatment as well as in the aquatic environment (e.g. via UV radiation) and in oxidative processes used for disinfection in waterworks and recently in WWTPs [155, 196, 197]. The (eco)toxicity of metabolites and transformation products (TPs) can be comparable or even higher than those of the original pharmaceuticals [173]. Although long term effects of these pollutants on aquatic organisms are still broadly unknown, high toxicities for some therapeutic classes such as endocrine disruptor have already been reported [198, 199] and synergistic effects have even shown to increase environmental impacts [200, 201]. However, there is still a lack of robust analytical methods to monitor extreme polar pharmaceuticals, their metabolites and TPs in aqueous environmental matrices. Extreme polar molecules are only minimally retained on commonly used reversed-phase HPLC columns and therefore require special chromatographic methods such as hydrophilic interaction chromatography (HILIC). 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 [202]. 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 [203–207]. Advantages of HILIC over reversed-phase liquid chromatography (RPLC) include the appreciable retention of polar to very polar analytes, low back pressure and higher compatibility with ESI due to elevated organic contents in the eluent [203]. Despite these appreciable advantages in comparison to RPLC, HILIC has rarely been employed for the analysis of environmental samples up to date. The main reasons for its little distribution are presumably more the lack of experience in the special requests of method development and troubleshooting in HILIC than limited capabilities. However, some HILIC methods were applied for the analysis of a given class of molecules: cytostatics [208], antibiotics [209], antidiabetic drugs [210], drugs of abuse [211, 212], organophosphorus pesticides [213] or aromatic amides [214]. Nonetheless, only few multi-methods exist [215] covering a limited range of compounds with similar polarities.

In this study we demonstrate the efficiency of HILIC for the analysis of extreme polar

analytes in environmental samples and provide a critical overview of the benefits and limitations of HILIC. In this framework, 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 were developed. The analytes were chosen due to their environmental relevance and to their elevated polarity (Table 2.1, see Table A.1 for structures) so that the results could indicate the applicability of HILIC for environmental analysis.

Table 2.1: List of selected analytes, application and log D and charge at pH 7.

Name	Application	Cas No	Formula	Log D at pH 7[a]	Charge at pH 7[a]
4-Acetamidoantipyrine	Metabolite of dipyrone [216]	83-15-8	$C_{13}H_{15}N_3O_2$	0.15	Neutral
4-Formylaminoantipyrine	Metabolite of dipyrone [216]	1672-58-8	$C_{12}H_{13}N_3O_2$	0.11	Neutral
4-Methylaminoantipyrine	Metabolite of dipyrone [216]	519-98-2	$C_{12}H_{15}N_3O$	0.77	Neutral
9-Acridine carboxylic acid	TP of carbamazepine [217]	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 [109]	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 [218]	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 [109]	1238210-10-0	$C_8H_8FN_3O_4S$	-3.88	Negative
Emtricitabine *S*-oxide	TP of emtricitabine [109]	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 [219]	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
Guanylurea	TP of metformin [220]	141-83-3	$C_2H_6N_4O$	-2.06	Neutral
N-acetyl mesalazine	Metabolite of mesalazine [221]	51-59-2	$C_9H_9NO_4$	-2.26	Negative
Oxipurinol	Metabolite of allopurinol [222]	2465-59-0	$C_5H_4N_4O_2$	-3.03	Negative
Paracetamol	Analgesic	103-90-2	$C_8H_9NO_2$	0.91	Neutral
Ranitidine	H2 receptor antagonists	66357-35-5	$C_{13}H_{22}N_4O_3S$	0.13	Zwitterion
Desmethyl ranitidine	Metabolite of ranitidine [221]	66357-25-3	$C_{12}H_{20}N_4O_3S$	-0.80	Zwitterion
Ranitidine *N*-oxide	Metabolite of ranitidine [221]	73857-20-2	$C_{13}H_{22}N_4O_4S$	-0.13	Zwitterion
Ranitidine *S*-oxide	Metabolite of ranitidine [221]	73851-70-4	$C_{13}H_{22}N_4O_4S$	-1.17	Zwitterion

[a] https://chemicalize.com/

2.2 Material and methods

2.2.1 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-d_3, abacavir-d_4, acyclovir-d_4, bisoprolol-d_7 hemifumarate, clindamycin-d_3, diatrizoate-d_6, emtricitabine-^{13}C,$^{15}N_2$, gabapentin lactam-d_6, guanylurea-$^{15}N_4$ hydrochloride, lamivudine-^{13}C,$^{15}N_2$, paracetamol-d_4, acesulfame-d_4

potassium salt and oxipurinol-^{13}C,$^{15}N_2$ 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-d_{10} 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.

2.2.2 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 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.

2.2.3 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 degasser 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 A 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% acetic acid). Additionally, the influence of the increase of the equilibration time from 11 to 30 min was 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 IonDrive™ ion source. Electrospray ionization (ESI) with polarity switching was used. The MRM transitions and the substance-dependent parameters are described in Table A.2. 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.

2.2.4 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 A.3), 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, guanylurea 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 ng/L 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, ^{13}C or ^{15}N) 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 with a signal-to-noise ratio (S/N) >10 for the transition of quantification and a S/N >3 for the transition of confirmation 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.

2.2.5 Matrix effects

The matrix effects (ME) were determined according to Matuszewski et al. [223]. The samples were spiked after lyophilisation and the areas were compared with a matrix free standard solution of the same concentration.

$$ME[\%] = \left(\frac{Area\ of\ sample\ spiked\ after\ lyophilisation - Area\ of\ non\text{–}spiked\ sample}{Area\ of\ standard} - 1 \right) \times 100 \qquad (2.1)$$

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.

2.2.6 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.

2.3 Results and discussion

2.3.1 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 (Figure 2.1).

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 [204, 224]. They are often recommended to ensure the reproducibility of the chromatography [225] and to decrease the interactions of the analytes with the charged stationary phase [225, 226]. In our study, it was found that 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 (Figure 2.2). 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 A.1).

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

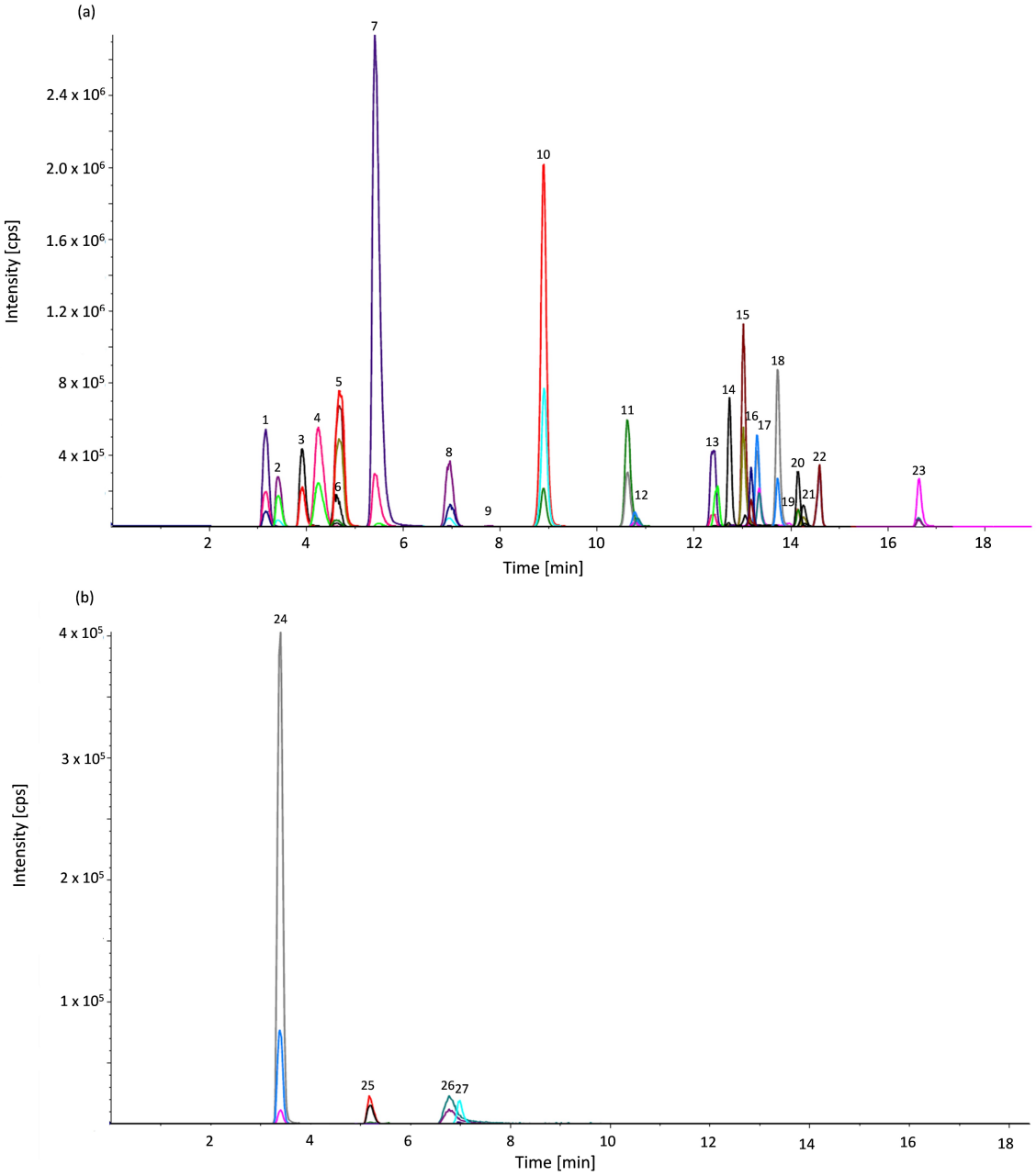


Figure 2.1: Superposition of MRM transitions of a 500 ng/L multi-standard. (a) Positive ionization mode. (b) Negative ionization mode. Conditions: HILIC Nucleodur (250 ×3 mm, 3 µm), eluent A (pH 3.3): 10 mM ammonium formiate, 0.1% formic acid, eluent B: acetonitrile/Milli Q, 90/10, v/v, 7.5 mM ammonium 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) guanylurea; (21) diatrizoate; (22) clindamycin sulfoxide; (23) ranitidine *S*-oxide; (24) acesulfame; (25) oxipurinol; (26) *N*-acetyl mesalazine; (27) abacavir carboxylate.

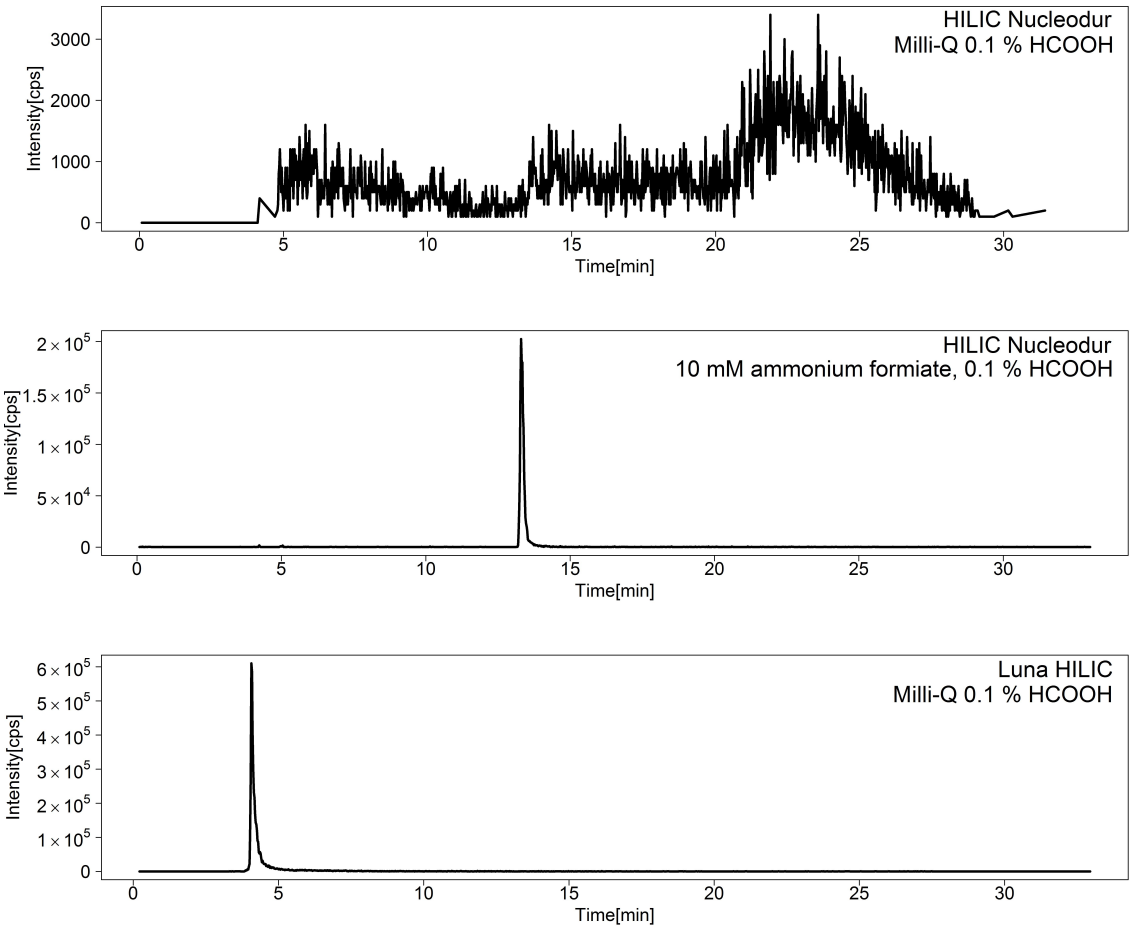


Figure 2.2: Influence of the composition of the aqueous eluent and the column on the elution of metformin. Conditions: (a) column: HILIC Nucleodur (250 x 3 mm, 3 µm), eluent A: 0.1% formic acid, eluent B: acetonitrile. (b) column: HILIC Nucleodur (250 x 3 mm, 3 µm), eluent A: 10 mM ammonium formiate, 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.

some cases, it affects also the charged state of the stationary phase [204, 224]. 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 [227–229]. Due to the extended equilibration time needed after the end of the HILIC chromatography run, one recommendation is to use an isocratic elution [230]. 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 the minimum to ensure reproducible retention times (Table A.4) 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 (Figure 2.3). This indicates that the system was still not fully equilibrated after 11 min. Thus, equilibration time is a critical parameter and has to be always the same between two runs. Especially during method development this important point can be easily neglected. By using an automatic sampler and blank runs at the beginning of a sequence constant equilibration times can be guaranteed. This illustrates the importance of a compliance of the exact and reproducible chromatographic conditions as well as the complexity of chromatographic mechanisms occurring in HILIC.

2.3.2 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 [220, 231]. 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 2-4 pH values in Milli-Q were investigated. Unfortunately, none of them showed the ability to simultaneously retain all or most of the selected analytes (Table 2.2, see Table A.5 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

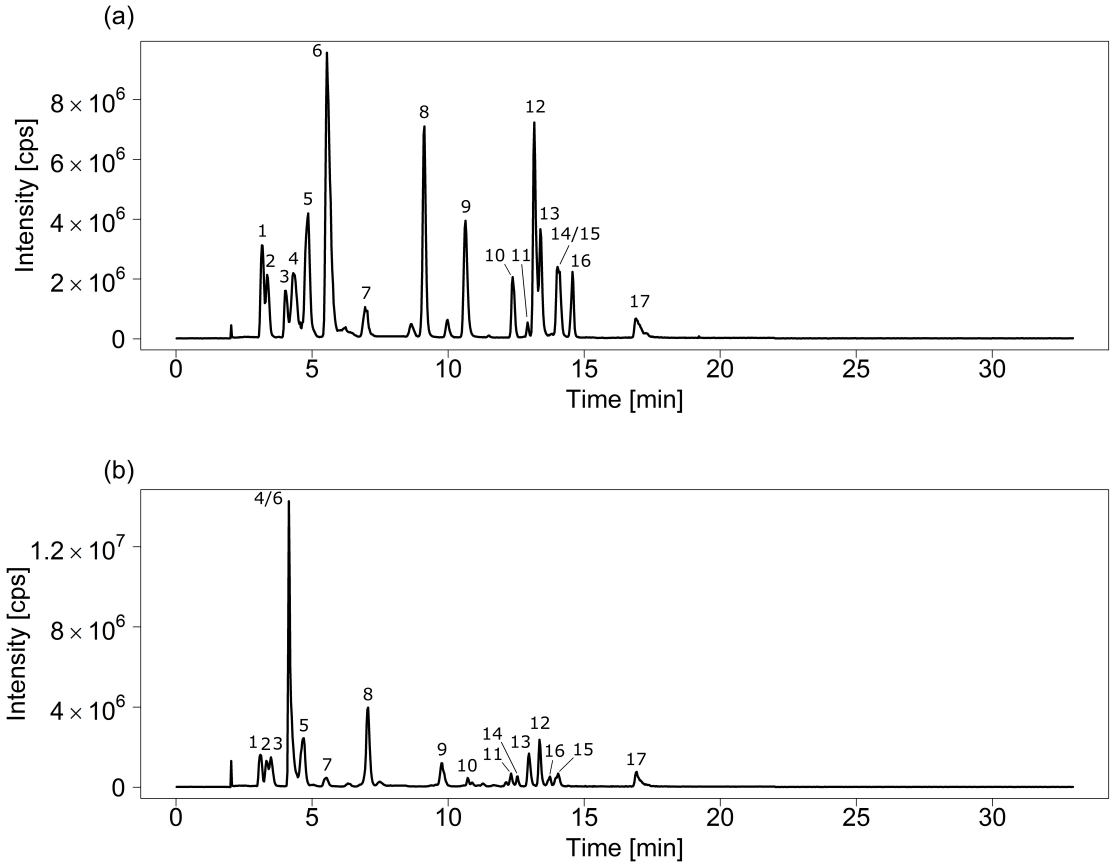


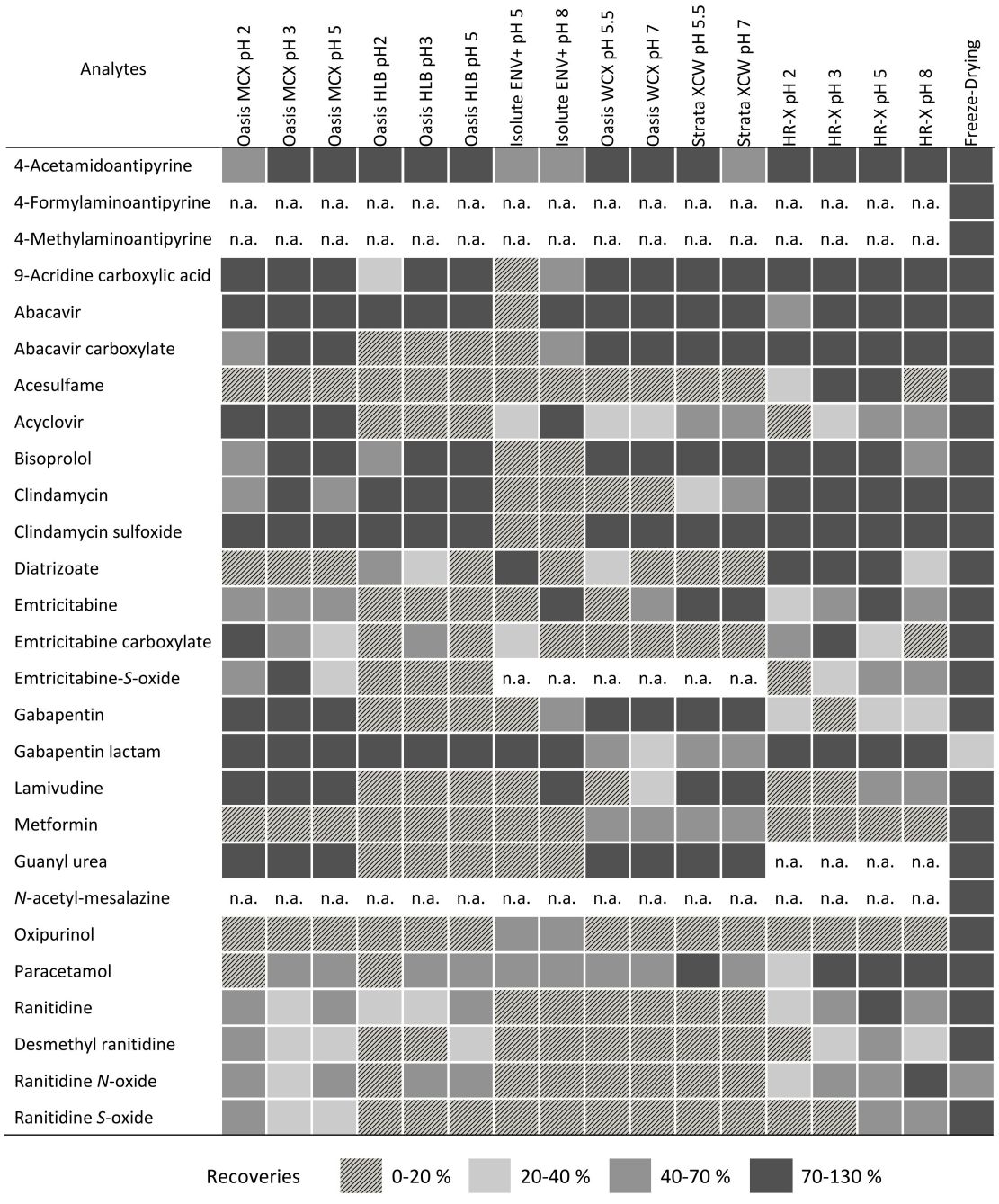
Figure 2.3: Comparison of 2 injections of a 2000 ng/L standard with the HILIC Nucleodur. (a) 11 min equilibration. (b) 30 min equilibration. Conditions: column: HILIC Nucleodur (250 x 3 mm, 3 µm), eluent A: 10 mM ammonium formiate, 0.1% formic acid, eluent B: acetonitrile/Milli Q, 90/10, v/v, 7.5 mM 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.

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 A.5). 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 2.2). This is probably related to a higher volatility in water with a low salt content, since almost quantitative 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. [232] but to the best of our knowledge, it is the first time that freeze-drying is used for sample preparation method prior to 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 a high throughput, with sufficient recoveries for most selected analytes (see Section 3.3).

2.3.3 Method performance

The method validation was carried out for four matrices: Milli-Q, groundwater, Rhine 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 A.6) 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 2.3). 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 Rhine water, only 100 ng/L and 1000 ng/L were

Table 2.2: Recoveries of the analytes with the different investigated sample preparation procedures (see Table A.5 for the exact recovery values).



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

Table 2.3: 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)		Groundwater	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
Guanylurea	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

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 11 analytes, no isotope labeled standards were available and for metformin and gabapentin a surrogate proton/deuterium exchange occurred during freeze-drying. Consequently, for metformin, gabapentin 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 2.4). 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 Rhine 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 (see Section 3.4).

Table 2.4: 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	Absolute	Relative	Absolute	Relative	Absolute	Relative	Absolute	Relative	Absolute	Relative	Absolute	Relative	Absolute	Relative	Absolute	Relative	Absolute	Relative
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 [a]	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
Clindamycin sulfoxide	83 ± 2	-	101 ± 11	-	102 ± 3	-	77 ± 6	-	91 ± 15	-	117 ± 40	-	94 ± 24	-	104 ± 4	-	87 ± 22	-
Diatrizoate [a]	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 [a]	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	-
Guanylurea [a]	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 [a]	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
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	-

[a] To correspond with the environmental concentrations all spiked levels were multiplied by 10 for these analytes. n.d.: not determined

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 [233]. For most substances, LOQ <10 ng/L were observed in groundwater and Rhine water (Table 2.3). 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-µg/L range.

2.3.4 Matrix effects

Matrix effects determined were relatively similar in the different matrices (Table 2.5). 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 (Figure 2.4 (a) and (b)). Appreciable signal enhancements were obtained between 3.8 and 5.6 min and 9.7 and 12 min.(4.7 min) and acyclovir (10.8 min) fall into one of these time windows (Figure 2.4 (c)). This is in conformity with the positive matrix effects observed in environmental samples.

Since 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 (Figure 2.5 (a)) correspond to the retention time of nitrate and chloride, respectively (Figure 2.5 (b) and (c), Table A.7). The salt concentrations of chloride and nitrate were estimated to be 0.5 mmol/L and 0.1 mmol/L, respectively. 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 (Figure 2.6, (a) and (b)). Several analytes such as metformin did not show any ion enhancement (Figure 2.6, (c)). 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 [231]. Iavarone et al.

Table 2.5: Matrix effect for the analytes in groundwater, Rhine water and WWTP effluent.

	Matrix effect [%]		
Analyte	Groundwater	Rhine water	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
Guanylurea	-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

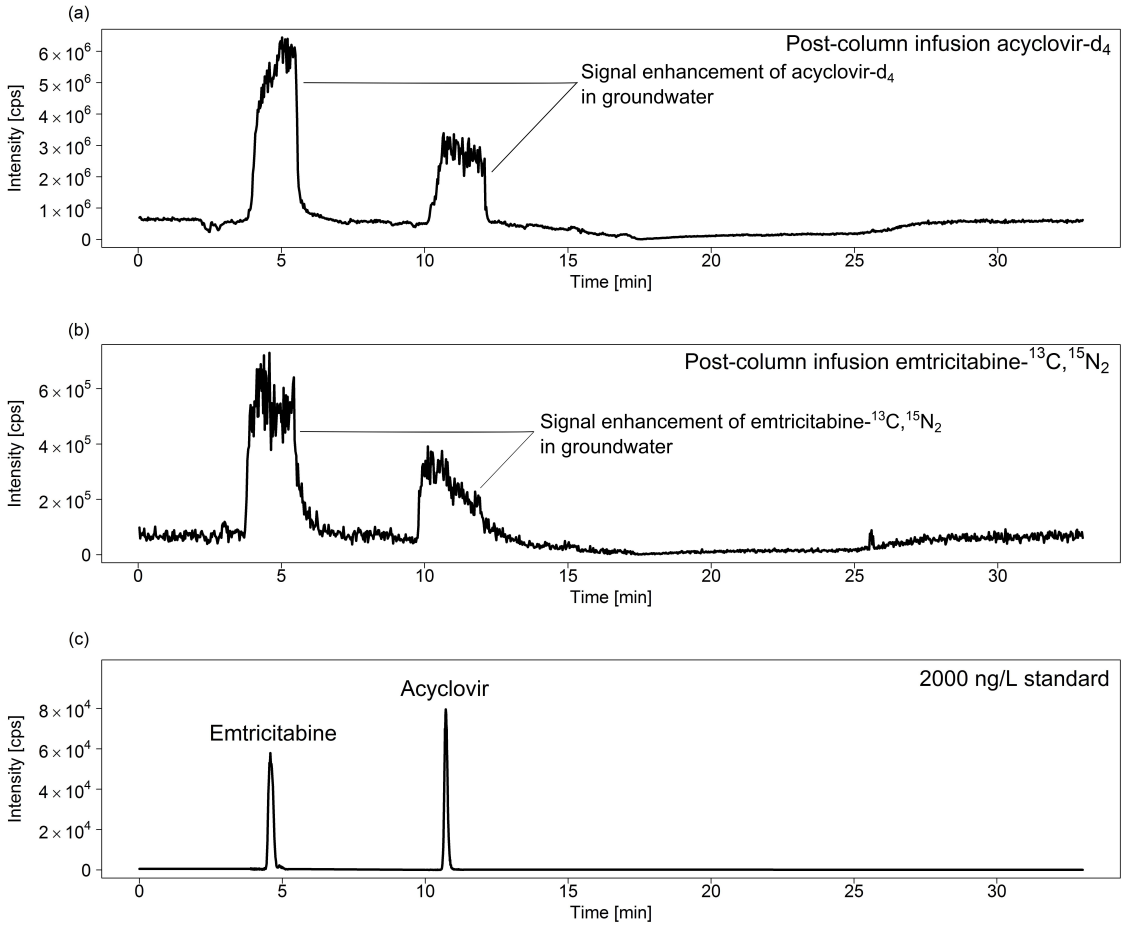


Figure 2.4: (a) Post-column infusion of 1 mg/L acyclovir-d_4 at 10 µL/min for HILIC-ESI-MS/MS analysis of a groundwater sample. (b) Post-column infusion of 1 mg/L emtricitabine-$^{13}C,^{15}N_2$ at 10 µL/min during HILIC-ESI-MS/MS analysis of a groundwater sample. (c) Superposition of MRM transitions 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.

[227] 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 NH_4Cl 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. In the standard solution, the sodium adduct could be clearly identified, while in the groundwater it was not detected anymore (Figure 2.7). 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.

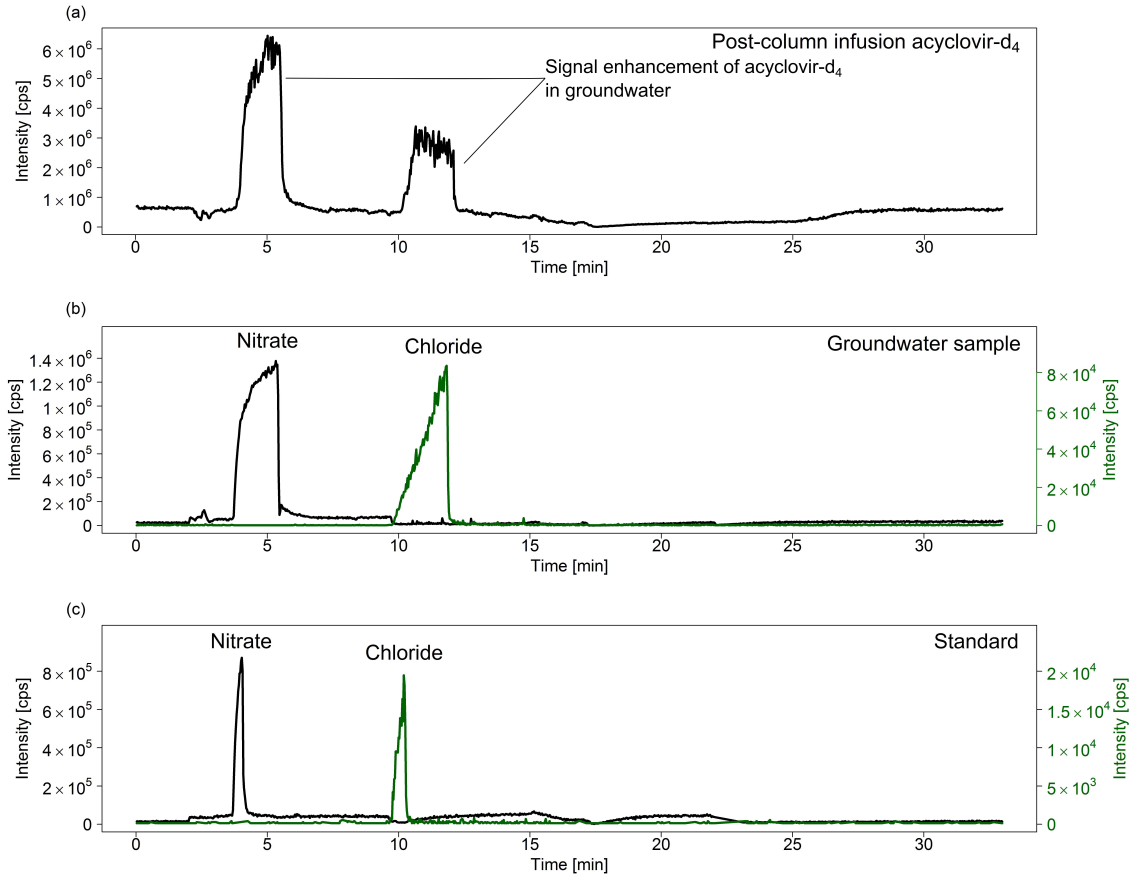


Figure 2.5: (a) XIC of acyclovir-d_4. Post-column infusion at 10 µL/min of 1 mg/L acyclovir-d_4 during the measurement of groundwater sample. (b) XIC of nitrate and chloride for a groundwater sample. (c) XIC of nitrate and chloride for a chloride and nitrate mixed 10 mg/L standard. Conditions: column: 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: 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.

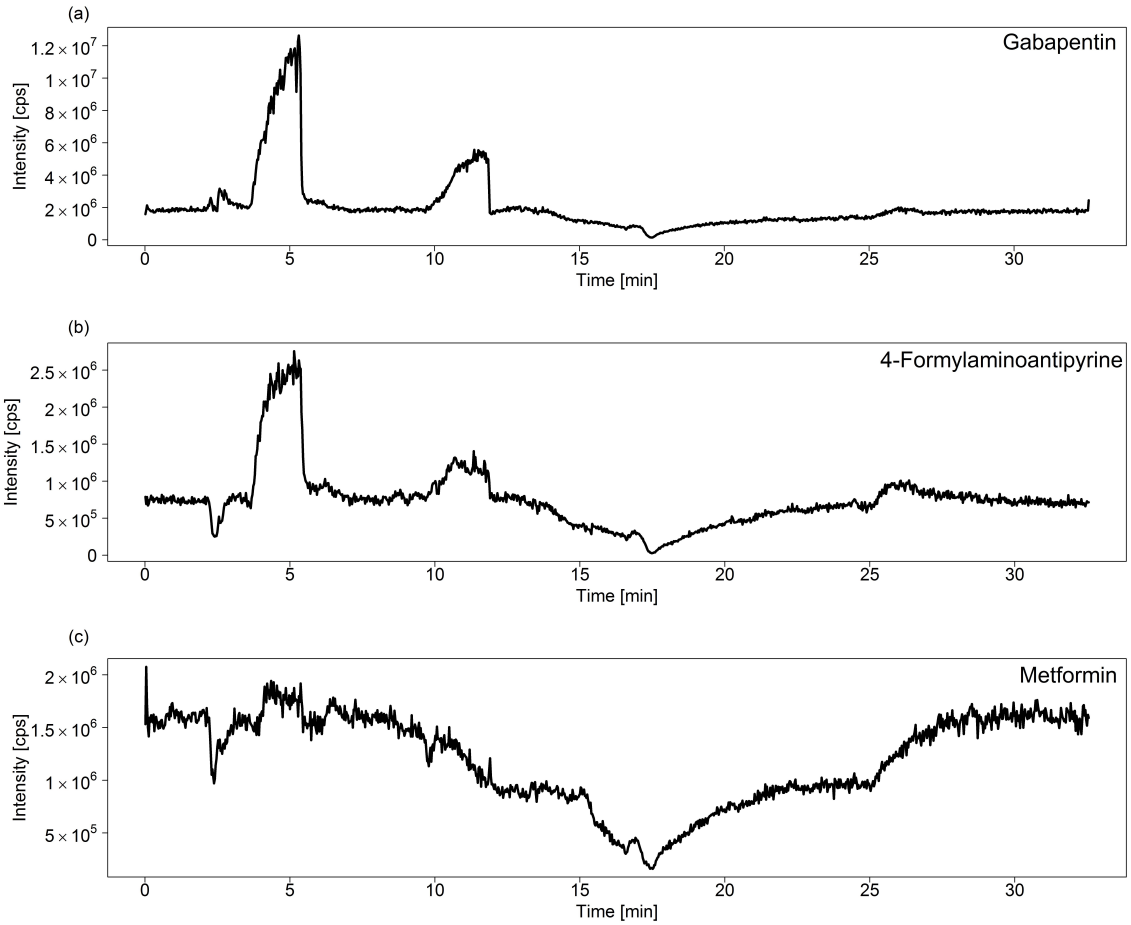


Figure 2.6: Post-column infusion of a 0.1 mg/L multi-standard at 10 µL/min during the measurement of a groundwater sample. (a) XIC of gabapentin. (b) XIC of 4-formylaminoantipyrine. (c) XIC of metformin. Conditions: column: HILIC Nucleodur (250 x 3 mm, 3 µm), eluent A: 10 mM ammonium formiate, 0.1% formic 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.

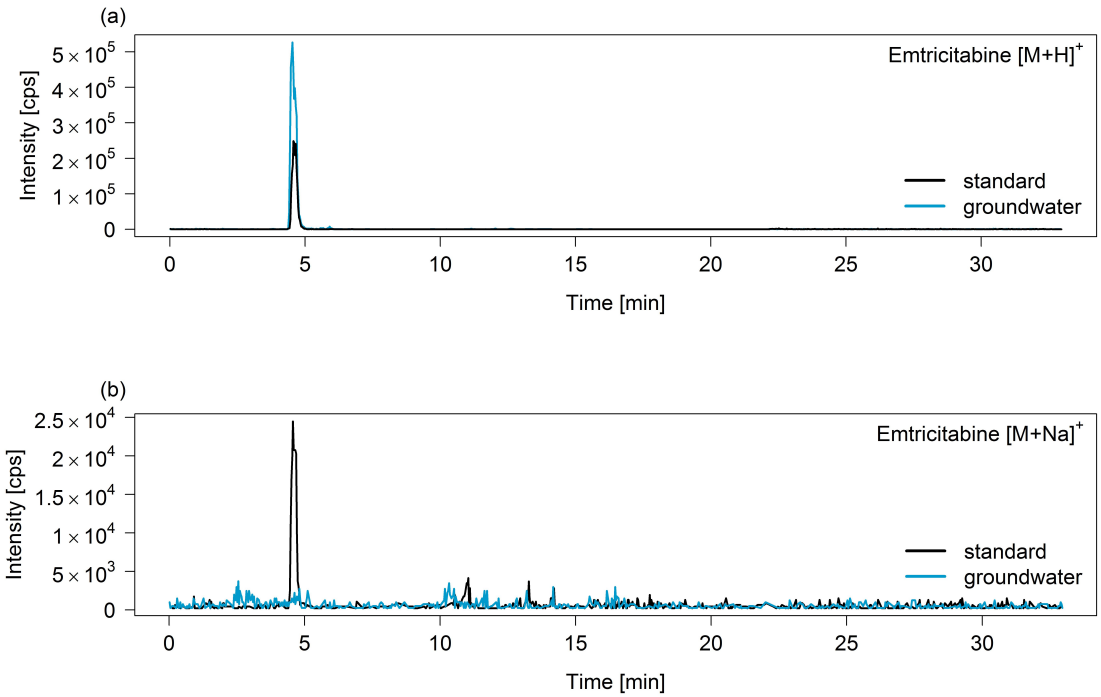


Figure 2.7: (a) XIC of the protonated ion of emtricitabine for a 1000 ng/L standard and a 1000 ng/L spiked groundwater sample. (b) XIC of the sodium adduct of emtricitabine for a 1000 ng/L standard and a 1000 ng/L spiked groundwater. Conditions: column: HILIC Nucleodur (250 x 3 mm, 3 µm), eluent A: 10 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.

2.3.5 Robustness

In comparison to RPLC, HILIC is known to be more sensitive to small changes of the chromatographic conditions [228]. It was investigated how even little modifications affect the chromatography. In HILIC, the composition of the sample diluent can strongly impact the chromatography [226, 229, 234]. 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 (Figure 2.8), while the analytes with higher retention times were less impacted. Thus, for half of the compounds (Table A.8) 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). Also slight variations of the acetonitrile/Milli-Q ratio of eluent B had significant effects on the retention times and the peak forms (Figure 2.9). 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% (Figure 2.9). 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 [230]. Since the chromatography was not impacted by an increase of the ammonium formiate concentrations, the buffer concentrations are obviously sufficiently high.

2.3.6 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 2.6). Detailed results are provided in the Supporting Information (Table A.9). In WWTP effluents, 24 of 27 analytes were detected. Guanylurea, the main transformation product of

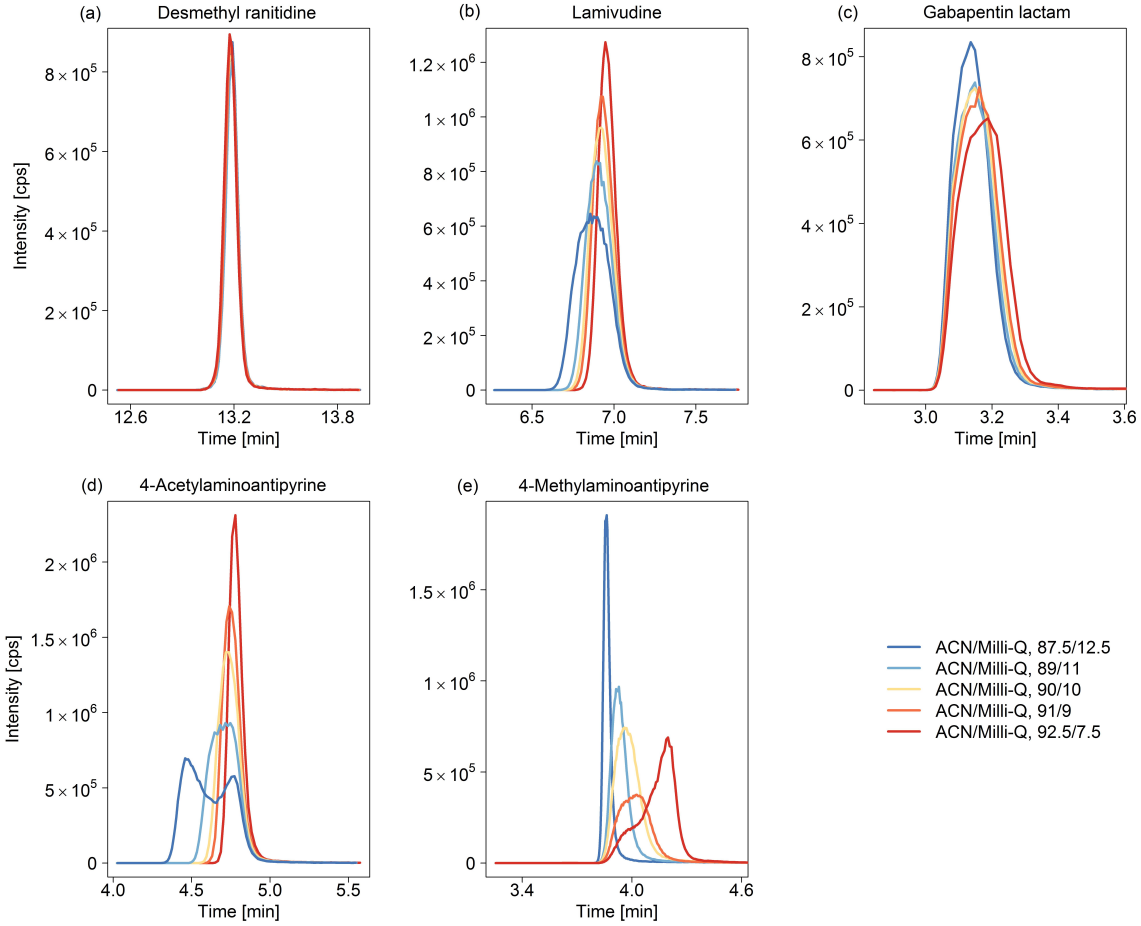


Figure 2.8: Retention times and peak forms of a 1000 ng/L multi-standard dissolved in different diluents: (a) XIC of desmethylranitidine, (b) XIC of lamivudine, (c) XIC of gabapentin lactam, (d) XIC of 4-acetamidoantipyrine, (e) XIC of 4-methylaminoantipyrine. Conditions: column: HILIC Nucleodur (250 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.

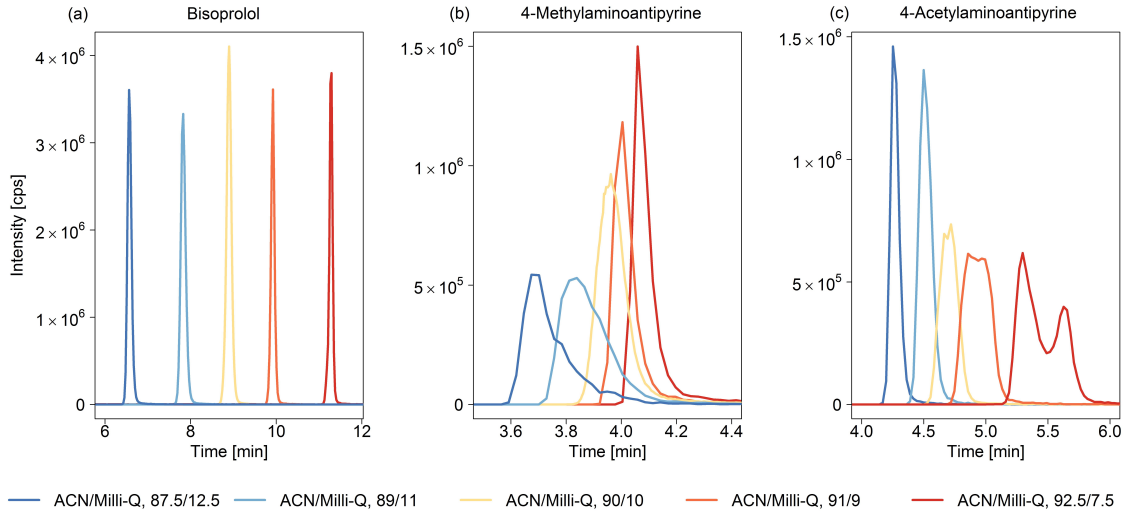


Figure 2.9: Retention times and peak forms of a 1000 ng/L multi-standard measured with eluent B containing different ratio acetonitrile/Milli-Q. (a) XIC of bisoprolol. (b) XIC of 4-acetamidoantipyrine. (c) XIC of 4-methylaminoantipyrine. Conditions: column: HILIC Nucleodur (250 x 3 mm, 3 µm), eluent 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.

metformin [220], showed with 110 µg/L the highest maximum concentration. Metformin, the most prescribed pharmaceutical in Germany in 2015 [235], and its transformation product have shown to be very persistent in the aquatic environment [210]. The measured concentrations (0.71-4.2 µg/L for metformin and 3.6-110 µg/L for guanylurea) are in accordance with those measured by Scheurer et al. (1.3-26 µg/L and 18-99 µg/L, respectively [210]). In addition to guanylurea 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 metabolites of the analgesic drug metamizole [216]. Gabapentin is an antiepileptic pharmaceutical which has been analyzed in the µg/L range in WWTP effluents and surface water [121]. Oxipurinol is the main metabolite of allopurinol, an anti-gout drug [222]. Metamizole, gabapentin and allopurinol belong to the most frequently consumed pharmaceuticals in Germany (572, 79 and 134 t in 2015, respectively [235]). Diatrizoate is used at high doses (several mg/day) is only minimally metabolized and is persistent under aerobic conditions [236]. Acesulfame is widely used in food and beverages and is mainly excreted unmetabolized [237]. 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 [238] and biodegradation [109]. However, its main metabolite and biotransformation product, abacavir carboxylate [109, 239] 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 [240, 241], making its detection in the environment relatively rare in spite of its high consumption level (56 t in 2009 [242]). *N*-acetyl-mesalazine (*N*-acetyl-5-aminosalycilic acid) is the main metabolite of mesalazine (excreted from 8 to 77% [121]) which belongs to the ten most prescribed pharmaceuticals in Germany (106 t in 2015 [235]). High removal rates of mesalazine in WWTPs have already been reported [243] 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.

Table 2.6: 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 frequence [%]	Average [µg/L]	Median [µg/L]	Maximal [µg/L]	Minimal [µg/L]	Detection frequence [%]	Average [µg/L]	Median [µg/L]	Maximal [µg/L]	Minimal [µg/L]	Detection frequence [%]	Average [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	0.016	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	0.0007	<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	100	0.12	0.091	0.25	0.047	33	0.006	<0.002	0.07	<0.002	0	<0.001	<0.001	<0.001	<0.001	<0.001
Bisoprolol	100	0.30	0.30	0.41	0.19	89	0.03	0.016	0.2	<0.001	7	0.0002	<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	<0.001	0.01	<0.0001	<0.0001
Clindamycin sulfoxide	100	0.28	0.27	0.39	0.2	89	0.039	0.046	0.12	<0.001	33	0.0012	<0.001	0.01	<0.001	<0.001
Diatrizoate	88	7.3	6	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	0.00062	<0.001	0.0039	<0.001	<0.001
Emtricitabine carboxylate	100	0.33	0.28	1	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	0.15	0.14	0.38	<0.2	0	<0.05	<0.05	<0.2	<0.05	13	0.0028	<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
Lamivudine	50	0.021	0.016	0.058	<0.02	0	<0.005	<0.005	<0.02	<0.005	13	0.00023	<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
Guanylurea	100	65	88	110	3.6	89	1.6	1.1	3.4	<0.02	7	0.0021	<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	0.0006	<0.005	0.004	<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

2.4 Conclusion

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 standards are available for quantification, either the co-eluting anions have to be removed prior to 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 ground water and drinking water. However, the 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. Due to the co-elution of the analytes with high concentrations of anions (chloride, nitrate), the use of appropriate labeled internal standards is required for those 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 hardly retention on conventional stationary phases can be chromatographic separated and quantified in the lower ng/L range. To the best of our knowledge, this is the first time that a multi-analyte HILIC method for such a wide polar range of analytes (Table 2.1) in environmental samples has been described by using freeze-drying and detection by tandem MS detection. 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.

Acknowledgments

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3

Spatial distribution and temporal trends of pharmaceuticals sorbed to suspended particulate matter of German rivers

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Abstract

Although several studies confirmed a wide distribution of pharmaceuticals in rivers and streams, a limited knowledge is available about the partitioning of pharmaceuticals between the water phase and suspended particulate matter (SPM). To close this gap of knowledge, we developed and validated a sensitive and high throughput analytical method for the analysis of 57 pharmaceuticals, 42 metabolites and transformation products (TP) as well as the artificial sweetener acesulfame sorbed to SPM. The method was based on pressurized liquid extraction (PLE) followed by a clean-up via solvent exchange and detection via direct injection-reversed phase LC-MS/MS and freeze-drying-HILIC-MS/MS. Freundlich isotherms were determined for 90 analytes. All showed a linear sorption behavior. Distribution coefficients (K_d) ranged from 0.64 L/kg to 9300 L/kg. For 18 pharmaceuticals, K_d values were found to be above 100 L/kg. SPM of annual composite samples were analyzed to determine the pharmaceutical concentrations in the years between 2005 and 2015 at four sites of the river Rhine: Weil, Iffezheim, Koblenz and Bimmen as well as between 2006 and 2015 at one site of the river Saar, at Rehlingen. In these SPM samples, up to 61 of the 100 analytes were detected with concentrations up to 190 ng/g d.w. (dry weight) for guanylurea, a transformation product of the antidiabetic metformin. For most analytes, increasing concentrations were found along the length of the Rhine and higher concentrations were measured in Rehlingen/Saar. Normalization of the data with the antiepileptic drug carbamazepine as an intrinsic tracer for municipal wastewater indicated possible industrial discharges for four analytes. For most pharmaceuticals, the annual concentrations exhibited a good correlation with the consumption volumes in Germany.

3.1 Introduction

Each year, large quantities of pharmaceuticals are consumed world-wide, excreted via urine and feces into wastewater and thus reach wastewater treatment plants (WWTPs). The original pharmaceuticals, the human metabolites or on-site (e.g. sewer, WWTP) formed TPs are discharged via WWTP effluents in the receiving rivers and streams [196, 244]. Their presence in the urban water cycle is not only a potential risk for aquatic organisms [199] and the ecosystems [245], but also for drinking water quality [246] and thus for human health.

Once in the aquatic environment, micropollutants partition between the water phase and suspended particulate matter (SPM) [247]. Sorption reduces the mobility of micropollutants in the aquatic environment, modifies their bioavailability and limits the biodegradation [248]. As a consequence, knowledge about sorption affinities is crucial to understand the interactions between environmental compartments and to predict the loads of micropollutants sorbed on SPM. The sorption of pharmaceuticals on suspended particulate matter (SPM) has been much less investigated than that of more hydrophobic micropollutants such as PCBs or PAHs. The few published studies [249–253] concentrated on a limited number of selected pharmaceuticals, frequently, the ones with the elevated contamination levels detected in the water phase, and not necessarily those with the higher sorption potential.

While more hydrophobic pollutants such as PAHs and PCBs were broadly investigated in both SPM and sediment, pharmaceuticals have mostly be analyzed in sediment [254, 255]. The studies determining pharmaceuticals concentrations sorbed to SPM are scarce and are mainly focused on illicit drugs [256, 257] or analyzed only a limited number of pharmaceuticals [150, 258–260]. To date, two studies report the analysis of a higher number of pharmaceuticals. Da Silva et al. [253] analyzed SPM of Ebro river basin (Spain) and detected up to 31 of their 43 targeted pharmaceuticals with concentrations up to 571 ng/g d.w.. Aminot et al. [261] investigated 53 pharmaceuticals in a periurban river of Bordeaux (France) and detected up to 22 of 53 selected pharmaceuticals with concentrations up to 150 ng/g d.w. in SPM downstream of a WWTP. However, these studies did not include pharmaceuticals recently introduced to the market such as sitagliptin or aliskiren. They are mainly focused on parent pharmaceuticals, whereas metabolites or TPs are rarely included.

In the past, several authors have tried with variable success to correlate measured water concentrations with prescription data [124, 262, 263]. However, these studies were always limited by the lack of data in particularly regarding human excretion and degradation in

WWTPs. To the best of our knowledge, a relationship was reported between the human consumption and environmental concentrations detected over several years.

In the current study, we developed a high-throughput and sensitive analysis method via LC-MS/MS detection for the quantification of 100 pharmaceuticals, metabolites and TPs (Table B.1) sorbed to SPM. The analytes were selected due to their environmental relevance as well as to cover a large range of polarity. To the best of our knowledge, more than 60 compounds were analyzed for the first time in SPM (Table B.2). The method could be easily extended to include further pharmaceutical compounds. The method was applied to evaluate the partition coefficients of the pharmaceuticals between water and suspended matter and to elucidate the spatial distribution and temporal trends of pharmaceuticals sorbed to SPM of 5 different sites in German rivers between 2005 (respectively 2006 for the Saar) and 2015.

3.2 Material and methods

3.2.1 Chemicals

LC-MS grade methanol, acetonitrile and formic acid (all Lichrosolv©) were purchased from Merck (Darmstadt, Germany). Ammonium formiate (LC-MS grade) was purchased from Fluka Analytical (Seelze, Germany), acetic acid (LC-MS grade) from Sigma-Aldrich (Munich, Germany), chemsolute quartz sand from Th. Geyer (Reningen, Germany) and calcium chloride from Merck. Milli-Q (18.2 MΩ.cm, Merck Millipore, Darmstadt, Germany) was used as ultrapure water.

The analytical standard and internal standard suppliers are listed in Tables B.3 and B.4. Individual stock solutions at 1 g/L were prepared for each analyte in appropriate solvents (mainly methanol). From these solutions, multi-standard solutions were prepared in methanol.

3.2.2 Environmental samples

3.2.2.1 Water samples

Thirteen 28-day composite samples from the River Rhine (km 590.3, Koblenz, Germany) were sampled in 2015 to compare them with annual composite SPM sample taken from the same sampling location in 2015.

3.2.2.2 SPM

For the determination of temporal trends, SPM were sampled at four different sites of the river Rhine, Weil (km 173), Iffezheim (km 333), Koblenz (km 590) and Bimmen (km 863) and at one site of the river Saar: Rehlingen (km 54) (Figure 3.1). The samples were obtained from the German Environmental Specimen Bank located in Schmallenberg, Germany. Sampling and preparation are described in detail by Schulze et al. [264]. Briefly, the samples were collected by sedimentation boxes, permanently deployed and placed 0.5 to 2 m below water surface. They were sampled each month and the SPM were subsequently shock-frozen in liquid nitrogen. At the end of the year, the samples of each month were combined to annual composite samples, freeze-dried and homogenized before storage in liquid nitrogen with a temperature below -150 °C.

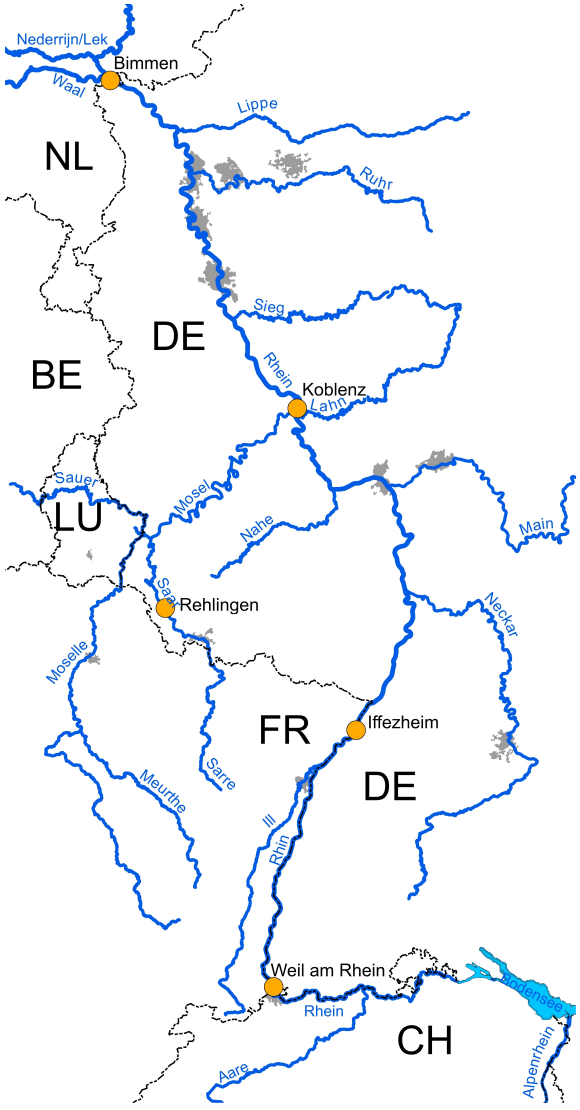

Figure 3.1: Sampling sites of the SPM

3.2.3 Consumption data

German annual pharmaceutical consumption quantities (2005-2015) originated from IQVIA Ltd (Frankfurt am Main, Germany) and were kindly provided by the German Environment Agency (UBA). These consumption quantities comprised all pharmaceuticals sold in pharmacies, including over-the-counter pharmaceuticals, as well as the pharmaceuticals quantities delivered in hospitals and medical practices.

3.2.4 Analytical methods

3.2.4.1 Water phase measurements

Water samples were filtered using a GF-6 glass fiber filters (pore size 1-3 µm, Whatman, GE Healthcare, Chicago, USA) and split to be measured both i) by direct injection reversed phase LC-MS/MS according to Hermes et al. [265] and ii) by HILIC-LC-MS/MS according to Boulard et al. [266]. Briefly, for the reversed phase method, 20 µL of an 0.01 mg/L internal standard solution was spiked to 1 mL sample prior to LC-MS measurement. For HILIC analysis, 20 µL of a 0.01 mg/L internal standard solution was spiked to 1 mL sample. Subsequently, the samples were frozen at -25 °C and freeze-dried with Christ Alpha 2-4 (Christ, Osterode am Harz, Germany), redissolved in acetonitrile/Milli-Q (90/10, v/v) prior to centrifugation and LC-MS measurement. The corresponding LC-MS parameters are described in detail in Table B.5.

3.2.4.2 SPM measurements

Sample extraction During method optimization, several extraction experiments were performed. First, ultrasound extraction (USE) and PLE were compared with methanol/Milli-Q (1/1, v/v) as an extraction solvent. For USE, three 15 min extraction cycles were run whereas for PLE three cycles with a static time of 15 min were carried out at 80 °C. Different extraction solvents were tested: methanol, Milli-Q/methanol (1/3, v/v), Milli-Q/methanol (1/1, v/v) and Milli-Q/methanol (3/1, v/v) and Milli-Q. Milli-Q/methanol (1/1, v/v) was selected. The pH of the water phase was varied by addition of formic acid (0.2%, 2%, v/v) or ammonium hydroxide (1%, v/v). A variety of extraction temperatures (80, 90, 100, 110, 120 °C) were compared.

For the final analytical method, SPM was extracted by pressurized liquid extraction (PLE) using an ASE 350 (Thermo Fisher Scientific, Darmstadt, Germany). Exactly 0.5 g freeze-dried SPM was thoroughly mixed with quartz sand and poured into a 10 mL stainless steel extraction cell. Internal standards dissolved in methanol (60 µL of a 0.1 mg/L solution) were directly added to the cell and the solution was evaporated prior to extrac-

tion. The first extraction cycle was performed with methanol/Milli-Q (1/1, v/v; 15 min static time, 20 s purge time, 0% flush volume) and two subsequent cycles were applied with methanol/2% formic acid, (1/1, v/v; 15 min static time, 120 s purge time, 150% flush volume). The whole extraction was carried out at 100 °C and 100 bar using a heating time of 6 min.

Clean-up The PLE extracts were cleaned-up by solvent exchange. They were filled up to 30 mL with methanol/Milli-Q (50/50, v/v). An aliquot of 10 mL was evaporated to dryness under a gentle nitrogen stream using a TurboVap LV (Biotage, Uppsala, Sweden). The residues were thoroughly redissolved in 100 µL Milli-Q water. Subsequently, 900 µL acetonitrile was added to precipitate the co-extracted impurities. The sample extracts were centrifuged for 10 min at 6000 rpm (3420 g) with a Hettich Mikro 220R (Tuttlingen, Germany). For the reversed phase chromatography, 100 µL supernatant was diluted in 900 µL Milli-Q, whereas for separation with HILIC, 200 µL of the supernatant was diluted in 800 µL acetonitrile/Milli-Q (90/10, v/v).

LC-MS analysis To cover the broad polarity range of the analytes, two LC-MS/MS methods with different column types were utilized, i) reversed-phase, adapted from Hermes et al. [265] and ii) HILIC adapted from Boulard et al. [266]. They are described in detail in Table B.5 and MS-specific parameters are given in Tables B.3 and B.4.

3.2.5 Method performance and quantification

Quantification was performed by a 14-point calibration ranging from 0.5 ng/L to 5000 ng/L. The quantification was based on a linear regression with a 1/x weighting factor. If available, internal standards were used to compensate matrix effects (Table B.6). Data processing was performed with the software Multiquant^{TM} 3.0.2 (SCIEX, Darmstadt, Germany).

Instrumental precision was determined by repeated injections of extracts of 10 ng/g d.w. (correspond to 167 ng/L for RPLC analytes and 333 ng/L for HILIC analytes) and 50 ng/g d.w. (correspond to 833 ng/L for RPLC analytes and 1667 ng/L for HILIC analytes) spiked SPM 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: 10 ng/g d.w., 50 ng/g d.w. and 100 ng/g d.w.. As no uncontaminated SPM was available, the original analyte concentrations were substracted prior to the calculation of the recoveries. Relative recoveries were calculated by normalizing the area of the analytes with that of the corresponding isotope-labeled internal standard. Precision of the method

was determined by calculating the 95% confidence interval of four separate spiked extracts. The LOQ was defined as the concentration at which a signal-to-noise ratio (S/N) of 10 is reached for the MRM transition used for quantification and a S/N of 3 for the MRM transition used for confirmation. Matrix effects were determined by spiking samples after the extraction and comparing their analyte peak areas with those of a standard at the same concentration.

Time trends were plotted using the LOESS method according to the approach developed by Fryer et al. [267]. This method used a locally weighted regression smoother. LOESS curves and their pointwise 95% confidence intervals were plotted using the ggplot2 package of the software R 3.5.0 (CRAN).

3.2.6 Sorption experiments

Sorption experiments were performed largely according to OECD 106 [268] with freeze-dried sediments from the Rhine harbor Ehrenbreitstein at Koblenz (Rhine km 591.4; TOC 3.9%, see Table B.7 for the other characteristics). The sorption isotherms were determined by two sediment/water ratios (1:5 and 1:25) to ensure a distribution of the analytes between the liquid phase and the solid phase between 20% and 80%.

Exactly 50 mL of 0.01 mol/L $CaCl_2$ was equilibrated with 2 g (25:1 sediment/water ratio) or 10 g (5:1 sediment/water ratio) freeze-dried sediments for 24 h using an orbital shaker (Ika KS260, 200 min^{-1}). Analytes were spiked from 10 to 2000 ng in duplicate via a multi-analyte stock solution. The methanol content in the sample did not exceed 1%. The samples were then agitated again for 24 h in the orbital shaker. The time was sufficient to reach sorption equilibriums for the analytes (data not shown). Afterwards, both phases were separated and analyzed as described in section 3.2.4.

For all analytes, the mass balance could be determined due to the quantification of both sediment and water phases. Sorption isotherms were plotted for those 90 analytes showing closed mass balance (± 30%, data not shown). Five concentration levels were spiked covering three orders of magnitude. For each concentration level and each matrix, the average of the duplicate analysis was plotted. Data processing was performed with the software R 3.5.0 (CRAN). The pH of the water phase varied between 6.9 and 7.9.

3.3 Results and discussion

3.3.1 Analytical method

The developed analytical method allows for a sensitive simultaneous detection of 100 pharmaceuticals, metabolites and transformation products sorbed to SPM. LOQs ranged

from 0.09 (clopidogrel) to 11.7 ng/g d.w. (dehydrotramadol) (Figure B.1 (a), Table B.8). The accuracy of the method was determined at three different spiking levels: 10 ng/g d.w., 50 ng/g d.w. and 100 ng/g d.w.. Due to appreciable environmental concentrations present in SPM of German rivers, spiking levels at lower concentrations were not performed. At all spiking levels the recoveries ranged from 70 (acesulfame) to 127% (O-desmethyl metoprolol) except for 13 analytes which showed either slightly higher or slightly lower recoveries at one of the spike levels (Figure B.1 (b)). An acceptable precision was obtained with 95% confidence intervals ranging from 1% (acesulfame) to 29% (sitagliptin) for the majority of analytes (Table B.8). Matrix effects ranged from -77% (sertraline ketone) up to +365% (aliskiren) (Figure B.1 (c)). However, they are successfully compensated for by isotopically labeled surrogate standards, even for aliskiren (Table B.8). For more than 50 analytes a quantification without isotopically labeled standards would be sufficient (Figure B.1 (d)). In general, the achieved LOQs were comparable to those reported in literature utilizing a SPE clean-up [253, 256, 257, 261]. In this study, a solvent exchange was preferred as a clean-up due to its unspecificity and high throughput. An appropriate SPE material would be very difficult to find since the selected analytes exhibit a broad range of polarities. Thus, a reliable analytical method has been developed enabling the quantification of 100 pharmaceuticals, metabolites and transformation products sorbed to SPM.

For the optimization of the extraction method, PLE and USE (ultrasound extraction) were compared using methanol/Milli-Q (1/1) as extraction solvent. On this basis, PLE was selected, since all analytes except diclofenac and lamotrigine showed higher recoveries than with USE (Figure B.2 (a)).

For the optimization of the PLE extraction solvent, different ratios of methanol/Milli-Q were tested. Overall, best recoveries were obtained with methanol/Milli-Q (1/1) (Figure B.2 (b)). The addition of ammonium hydroxide to the extraction solvent did not provide any significant improvement (Figure B.2 (c)), while the addition of formic acid improved the recoveries of positively charged analytes but decreased those of others, in particular valsartan, sulfamethaxole and candesartan. Highest recoveries for the positively charged analytes were obtained with methanol/2% formic acid (1/1, v/v) (Figure B.2 (d)). As a consequence, in the final analytical extraction method, both methanol/Milli-Q (1/1) and methanol/2% formic acid (1/1, v/v) were applied. This combination ensured the highest overall extraction efficiencies (see Figure B.2 (e)). Extraction temperature was varied between 80 and 120 °C. In general, the extraction recoveries improved with higher temperatures. This was particularly the case for positively charged analytes such as citalopram, whose extraction recoveries increased from 32 ± 14% (80 °C) to 100 ± 26% (100 °C).

Certain analytes such as diphenhydramine or hydrochlorothiazide exhibited a thermal degradation at temperatures above 110 °C. As no significant improvement of the extraction efficiencies was observed above 100 °C, an extraction temperature of 100 °C was selected (see Figure B.2 (f)).

3.3.2 Sorption

All selected compounds exhibited almost linear sorption isotherms with Freundlich n values ranging from 0.87 to 1.17 (Table B.9). Hence, sorption affinity could be described by the partition coefficient K_d. To ensure reliable partition coefficients, we measured the analytes in both the water and sediment phases and calculated the partition coefficients only when the mass balance was closed within an uncertainty of ± 30%. These conditions were fulfilled by 90 analytes.

Due to the high difference in polarity and pK_a values, the K_d values ranged from 0.64 L/kg (acesulfame) to 9300 L/kg (diphenhydramine). The obtained Kd values were comparable with those reported in literature (Table B.10). Exact 41 analytes showed partition coefficients below 10 L/kg, for 31 the K_d ranged between 10 and 100 L/kg and for 18 analytes K_d above 100 L/kg were determined (Figure 3.2). K_d values, Freundlich affinity parameters Kf and exponents n, determined by plotting the sorption isotherms and the logarithmic sorption isotherms, are summarized in Table B.9.

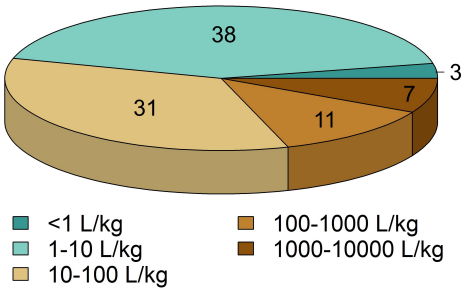

Figure 3.2: Distribution of the partition coefficients of the analytes

In general, positively charged analytes showed higher K_d values than the neutral and negatively charged substances (Figure B.3). All analytes with K_d>800 L/kg (sitagliptin) were positively charged at pH 7. Moreover, guanylurea, a polar and positively charged molecule (log D at pH 7: -3.9) exhibited a similar K_d value (14.0 ± 0.1 L/kg) as the considerably less polar but non-charged carbamazepine (10.0 ± 0.2 L/kg, log D at pH 7: 2.8). Franco et al. [269] reported a higher sorption affinity of protonated compounds than of neutral or negatively charged substances. It can be assumed that positively charged

analytes are susceptible to a higher sorption via cation exchange and surface complexation or cation bridging on negatively charged surface sites of organic matter and clay minerals [140, 270].

3.3.3 Concentration trends

3.3.3.1 Occurrence and spatial distribution of pharmaceuticals bound to SPM

Between 2005 and 2015, temporal trends of pharmaceuticals, as well as their metabolites and TPs were determined in SPM taken from five different sampling sites of German rivers (see Table B.11 for the TOC-values). The number of analytes and their concentrations detected in SPM increased with an increasing portion of wastewater. As a consequence, in the Saar at Rehlingen up to 61 analytes were found (about 15% wastewater[1]), while in SPM sampled along the Rhine, up to 24 analytes were detected in Weil (about 2% wastewater[1]) and up to 42 analytes in Bimmen (about 7% wastewater[1]). Maximal concentrations were determined for guanylurea, a TP of the antidiabetic metformin [220], with concentrations of 190 ± 6 ng/g d.w. in the Saar (Rehlingen) and 160 ± 6 ng/g d.w. in the Rhine (Bimmen). To the best of our knowledge, this is the first time that the occurrence of guanylurea in SPM is described. The analytes showing the highest concentrations (>10 ng/g) were either positively charged or more hydrophobic (log D >4). The few exceptions (acesulfame, leveritacetam acid and valsartan) correspond to substances whose consumption was relatively high (>100 t pro year (IQVIA Ltd. (2017) Midas© database, Frankfurt am Main, Germany)).

A comparison of the concentrations measured in SPM of the Saar and the Rhine helps to identify emissions from pharmaceutical industries, as those are very rarely located in the Saar catchment area in comparison to the Rhine catchment. Thus, municipal wastewater effluents are in nearly all cases the major source of pharmaceutical emissions in the Saar.

In order to distinguish the impact of municipal wastewater and direct or indirect industrial discharge, concentrations in SPM were normalized by the detected carbamazepine concentrations. The antiepileptic carbamazepine is well described in the literature as a marker of municipal wastewater due to its persistence and ubiquitous presence in rivers and streams containing appreciable portions of WWTP effluents [106, 271, 272].

The concentrations of sitagliptin and sotalol at the different sampling locations of the Rhine and in the Saar at Rehlingen are shown in Figure 3.3. For both pharmaceuticals, the concentrations increased along the Rhine due to an increasing wastewater proportion. In Rehlingen/Saar with the highest portion of WWTP effluents, the highest concentrations of

[1]Wastewater proportion were estimated from the wastewater proportion in Koblenz/Rhine (5%) [130] and the carbamazepine concentrations at the different sampling locations

the selected analytes were found. The concentrations normalized with the carbamazepine concentrations were relatively constant over the five sampling sites at the Rhine indicating that effluents of municipal WWTPs are the main source. A similar behavior was found for most analytes such as cetirizine or amisulpride.

In contrast to sitagliptin and sotalol, SPM concentrations of guanylurea, a TP of metformin, decrease along the Rhine from Weil to Koblenz (Figure 3.3). For example, in 2009, 119 ± 8 ng/g d.w. of guanylurea were measured in Rhine SPM at Weil in comparison to 71 ± 6 ng/g d.w. of guanylurea downstream in Koblenz. This difference cannot be caused by different consumption behaviors in the three major countries of the southern Rhine catchment area (Switzerland, France, Germany) since similar consumptions for metformin, the guanylurea precursor, were reported in 2009 with 9.6 g/capita for Switzerland, 12 g/capita for France and 12 g/capita for Germany [273]. Furthermore, despite a seven times higher wastewater proportion, similar concentrations were found in Rehlingen/Saar (124 ± 7 ng/g d.w.) and Weil/Rhine (119 ± 8 ng/g d.w.). This is particularly emphasized by the difference of carbamazepine normalized concentrations which reached a ratio of 106 ± 23 in Weil in comparison to a ratio of 17 ± 2 in Rehlingen/Saar. Therefore, we assumed that probably a significant industrial discharge of guanylurea, upstream Weil is causing the different ratios. Contrary to guanylurea, the concentrations of its precursor, metformin are proportional to the wastewater proportion (Figure 3.3). The guanylurea/metformin ratio decreases strongly along the Rhine varying for example in 2009 from 9.8 in Weil to 3.6 in Bimmen. Two possible explanations may explain this observation: i) metformin is quantitatively transformed to guanylurea in the WWTPs through which the discharge occurs. This is however relatively unlikely since quantitative transformation of metformin to guanylurea was to date only observed during long-term laboratory experiments [274]. ii) The discharged guanylurea did not originate from metformin, but from other precursors. The existence of other precursors than metformin for guanylurea was already hypothesized by Tisler et al. [275] to explain high guanylurea concentrations in WWTP effluent. They consider that guanylurea may have been formed from additional biguanide precursors, in particular from disinfectants.

Since 2007, fluoxetine exhibited significantly lower concentrations in Koblenz SPM (5.0 ± 0.3 ng/g d.w.) than in Weil (13 ± 1 ng/g d.w.). Fluoxetine consumption varied strongly between the countries of the Rhine catchment area with 0.032 g/capita in Switzerland, 0.062 g/capita in France and 0.012 g/capita in Germany [273]. Assuming a uniform pharmaceutical consumption of the people from the three countries, Swiss inhabitants have consumed about 240 kg fluoxetine up to Weil, whereas French and Germans inhabitants have consumed 317 kg fluoxetine between Weil and Koblenz (based on catchment area

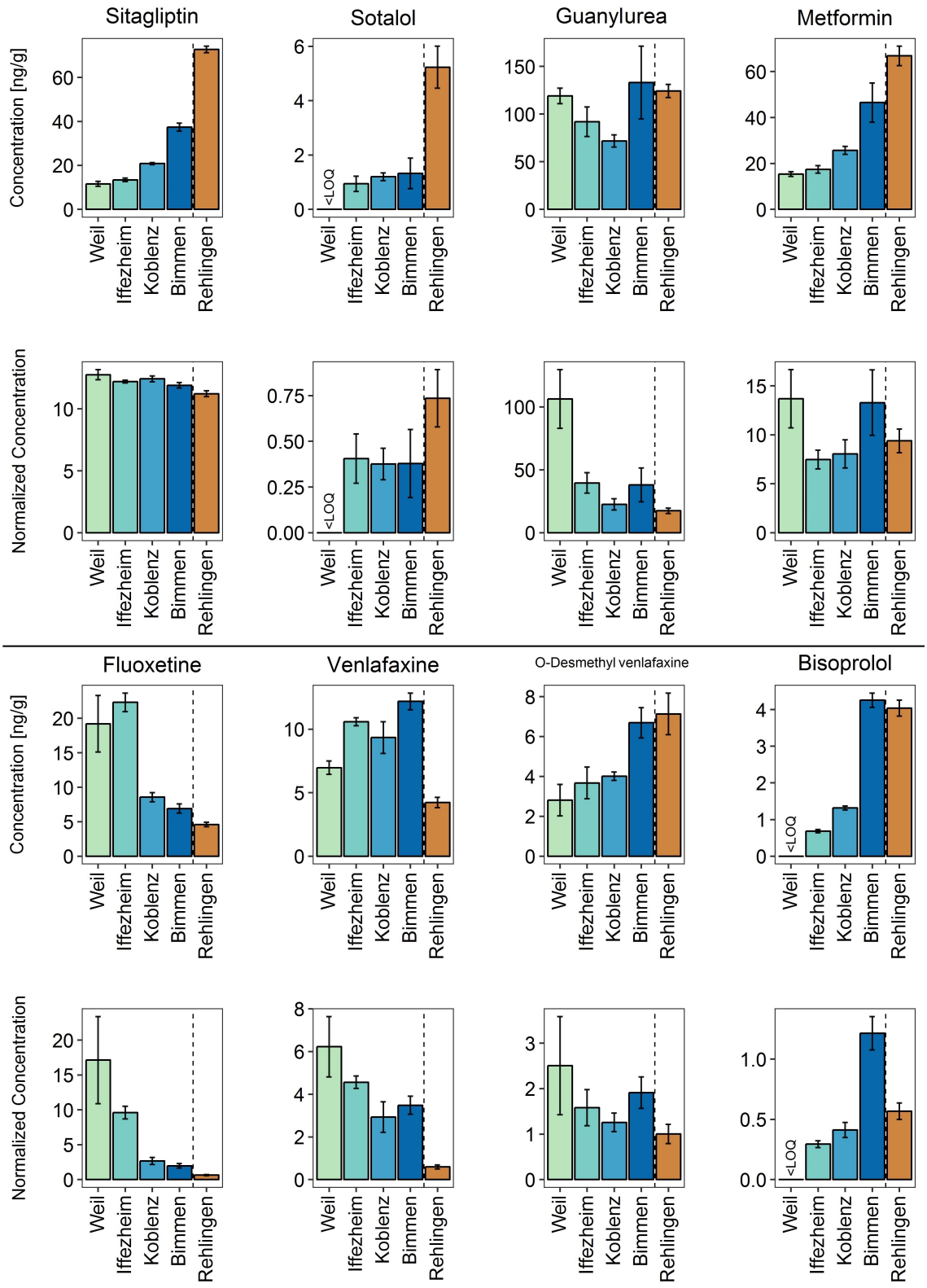

Figure 3.3: Concentrations and normalized concentrations (against carbamazepine) for sitagliptin, sotalol, guanylurea, metformin, fluoxetine, venlafaxine, O-desmethyl venlafaxine and bisoprolol at the different sample sites for the year 2014 for sitagliptin and 2009 for the others analytes. Error bars correspond to the 95% confidence interval.

population reported by Schlüsener et al. [130]). Thus, since the fluoxetine discharge between Weil and Koblenz is adding to the one consumed before Weil and that the dilution factor for fluoxetine between Weil and Koblenz is about 0.6, similar concentrations should be expected in Koblenz in comparison to Weil. Environmental dissipation is unlikely to play an important role since i) fluoxetine is believed to be persistent in surface water [276], ii) in 2006, similar concentrations were indeed observed in Weil (2.2 ± 0.4 ng/g d.w.) and Koblenz (2.0 ± 0.2 ng/g d.w.) (Figure B.4). Consequently, it can be hypothesized that higher concentrations of fluoxetine in SPM measured in Weil since 2007 are caused by specific industrial discharges of fluoxetine upstream of Weil. Moreover, from 2007 to 2014, fluoxetine shows higher concentrations in Weil (Rhine) than in Rehlingen (Saar) in spite of a much higher wastewater proportion of the river Saar. This further supports an additional industrial emissions of fluoxetine upstream of Weil.

Venlafaxine concentrations did also not correlate with the wastewater proportion since normalized concentrations were significantly higher in the Rhine than in the Saar (Figure 3.3). Furthermore, the ratio of O-desmethyl venlafaxine to venlafaxine was much lower in Rhine (0.3 in Weil in 2006) compared to the Saar (1.7 in Rehlingen in 2006). This also indicates that industrial emissions upstream of Weil contributed to a significant extent to the venlafaxine concentrations in the rivers.

Since bisoprolol was not detected in SPM taken in Weil, it is probably not used in appreciable quantities in Switzerland. Bisoprolol concentration increased significantly in Bimmen (Figure 3.3). In particular, between Koblenz and Bimmen a strong increase of the carbamazepine normalized concentration was observed with a ratio increasing from 0.4 to 1.2. This might be an indication for an additional input, probably by industrial discharge.

3.3.3.2 Temporal trends

For most pharmaceuticals, similar trends of the concentrations were observed at the different sampling locations from 2005 to 2015. The trends of four pharmaceuticals are shown in Figure 3.4 as examples. The temporal trends for the other analytes are illustrated in Figure B.5. These four substances are representatives for the diversity of pharmaceuticals that were detected in SPM, i.e. medium polar to apolar substances such as carbamazepine or telmisartan but also polar positively charged analytes such as sitagliptin or aliskiren. Sitagliptin and aliskiren were already detected in Rhine SPM (Bimmen) the year after their market authorization (2006) with concentrations of 0.40 ± 0.07 and 5.0 ± 0.4 ng/g d.w., respectively (Figure 3.4). Sitagliptin concentrations have strongly increased between 2006 and 2015 up to 49 ± 3 ng/g d.w. in 2015 in Bimmen. At the same sampling loca-

tion, aliskiren concentrations have increased up to 24 ± 3 ng/g d.w. in 2011 and declined subsequently down to 11 ± 1 ng/g d.w.in 2015. In the Rhine, telmisartan concentrations have increased until 2010 and were then relatively constant until 2015, nevertheless attaining 28 ± 2 ng/g d.w. in Bimmen/Rhine. In Rehlingen/Saar, telmisartan concentrations increased steadily between 2006 and 2015 reaching 120 ± 30 ng/g d.w. in 2015. Carbamazepine concentrations have decreased between 2005 and 2015 in all locations being in 2015 as low as 0.7 ± 0.2 ng/g d.w. in Weil/Rhein, corresponding to a concentration decrease of 65%.

If the concentrations of metabolites and TPs correlated mostly with those of their parent drugs (Figure B.5, Table B.12 for Pearson coefficients and p-values), there was most likely no major industrial discharge of their precursors upstream the sampling sites. For instance, desmethylcitalopram showed at each sampling location an excellent correlation with citalopram concentrations (Figure B.6, all Pearson coefficients >0.95, all p-values <0.005), while O-desmethyl venlafaxine did not correlate with the concentrations of its precursor at Weil/Rhine and Iffezheim/Rhine (Pearson coefficients <0.35, p-values >0.29) but does at Rehlingen/Saar (Pearson coefficient=0.99, p-value<0.005). Moreover, at Koblenz/Rhine and Bimmen/Rhine, its concentrations correlated to a lesser extent than at Rehlingen/Saar (Table B.12). This is a strong argument indicating an industrial discharge of venlafaxine in Switzerland as already stated in section 3.3.3.1. TPs having several precursors did not show a correlation with their parent drugs. This was for example the case for acridone and 9-carboxyacridine which can be formed from oxcarbamabazepine, 10,11-dihydroxy-10,11-dihydrocarbamazepine, 10,11-dihydro-10-hydroxycarbamazepine, in addition to carbamazepine [277]. Guanylurea also did not correlate with the metformin concentrations at any sampling locations (Table B.12), probably due to an industrial discharge as stated above.

It has to be noted that the concentrations of many pharmaceuticals correlated well with the annual consumed quantities in Germany (Table B.13 for Pearson coefficients and p-values). This was in particular the case for SPM from Koblenz and Bimmen, where respectively 52% and 63% of the analytes detected every year correlated (p <0.05, Pearson coefficients >0.6) with the German consumption. At these locations, the influence of different prescription behaviors in Switzerland and France can be assumed as negligible, since the proportion of effluents from German WWTPs increases with distance from Weil/Rhine. For carbamazepine, telmisartan, sitagliptin and aliskiren for example, excellent correlations could be determined at all sampling locations at the Rhine with Pearson coefficients >0.79 and p-values <0.005 (Figure B.7). For the compounds correlating well with the consumption data (Pearson coefficient >0.6, p-value <0.05), the parameters of

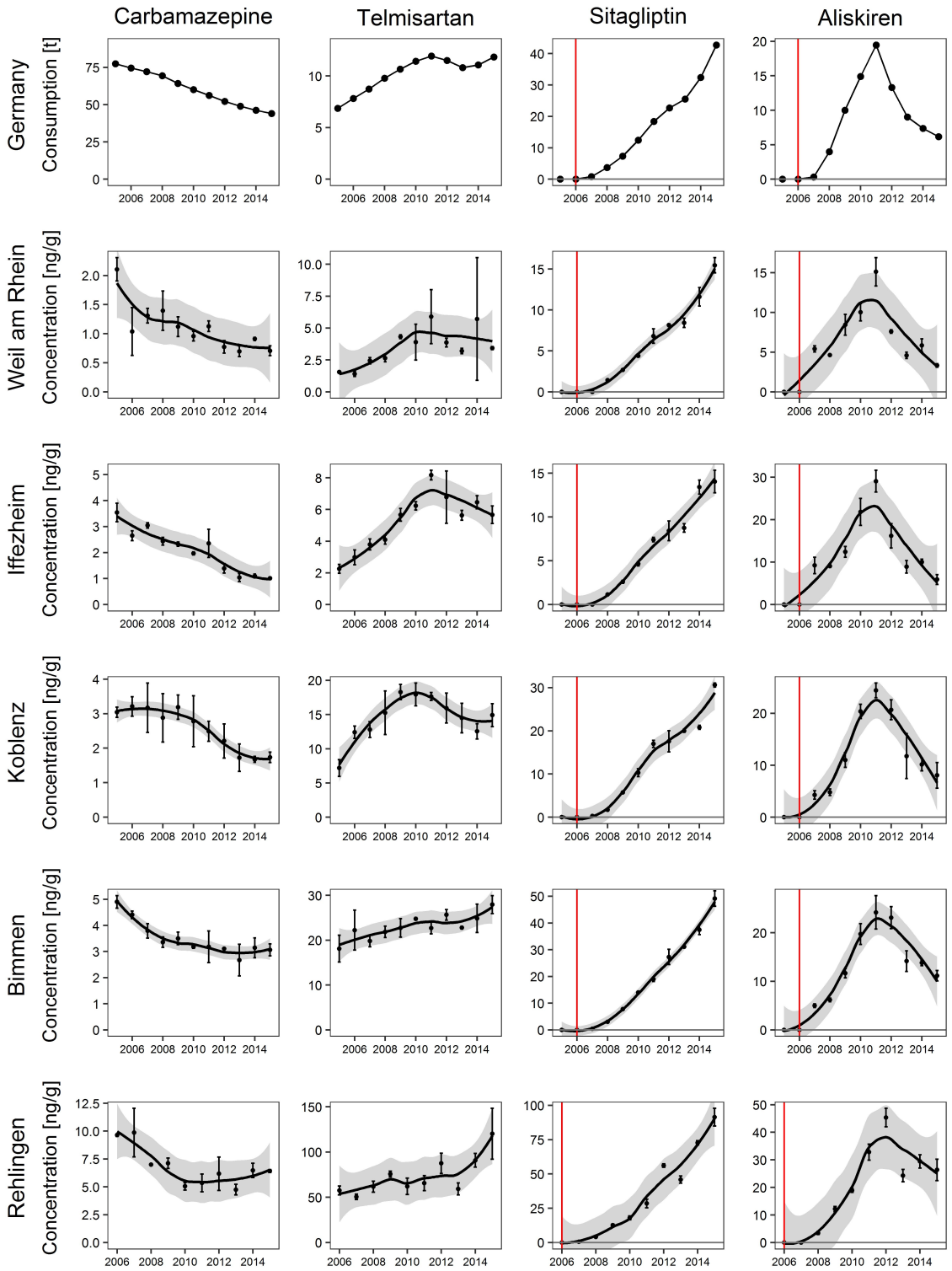

Figure 3.4: Consumption and temporal concentration trends of carbamazepine, telmisartan, sitagliptin and aliskiren in SPM from the sampling sites Weil am Rhein (Rhine, km 173), Iffezheim (Rhine, km 333), Koblenz (Rhine, km 590), Bimmen (Rhine, km 863), Rehlingen (Saar, km 54). The grey shaded area corresponds to the pointwise 95% confidence interval of the LOESS function. Measurements were performed in triplicate and the error bars correspond to the 95% confidence interval. Consumption data: IQVIA Ltd. (2017) Midas© database (Frankfurt am Main, Germany).

the linear correlation could be determined (Table B.14 for all parameters). Based on these linear relationships, the concentration in SPM can be predicted from its consumption. This is very interesting because these relationships consider parameters such as excretion, average WWTP degradation and partly surface water degradation which are very difficult to predict from literature data in particularly for large scale scenarios.

However, for certain pharmaceuticals, there were no significant correlations between their SPM concentrations and their German consumption. This was the case for pharmaceuticals whose German annual consumption varied only slightly between 2005 and 2015. This is probably due to a higher influence of Switzerland and French consumption variation. Fluoxetine and venlafaxine also showed relatively low correlations with the consumption in Germany, particularly in Koblenz what is coherent with the presence of an industrial discharge, postulated above.

Metformin concentrations did not correlate with the consumption trends (negative Pearson coefficients at each sampling location), for example in Bimmen the concentration in SPM has decreased from 64 ± 6 ng/g d.w. in 2005 to 37 ± 4 ng/g d.w. in 2015, whereas the German consumption has doubled in the same period. A conceivable explanation is an adaptation of the microbial population of the WWTPs to metformin causing increasing removal rates. Adaptation of the microbial population of the WWTPs was already observed for acesulfame which is present in similar concentrations to that of metformin in effluent, e.g. concentrations ranging from a few µg/L to hundreds µg/L [237, 271, 275] and related to an adaptation of the microbial sludge community caused by the high environmental concentrations [237]. Moreover, adaptation of the microbial population to metformin degradation was already observed at the laboratory scale by Scheurer et al. [210].

3.3.4 Relevance of SPM analysis

As the water quantity of the Rhine is about 50 000 times higher than that of SPM (concentrations ca. 20 mg/L), for all substances with $K_d \leq 500$ L/kg the sorbed fraction was negligible (<1%) in comparison to the dissolved percentage.

However, for long-term trend analysis an integrative sampling is essential. Sedimentation boxes enable continuous sampling over a longer period, while a continuous sampling of the water is very challenging. The methodology for the conservation of the SPM samples over a long period is well established, while this is not the case for water samples. The stability of the SPM samples is ensured by maintaining a consistent cold chain from the removal of SPM from the sedimentations boxes to their lyophilization using an active cooling system to limit degradation. Their subsequent conservation within liquid nitrogen

minimizes biological and chemical activities. The collection of SPM during one month raises issues about the stability of the analytes during this time. However, the good correlation of the analytes with their consumption data (performed in this study) indicates that the degradation is probably very low. This is also supported by the correlations observed between the concentrations of the parent pharmaceuticals with their metabolites. Thus, pharmaceutical concentrations at the same sampling location can be compared for a longer period. However, it cannot be ruled out that a comparison of two analytes is limited due to a different stability or the formation of non extractable residues. Thus, more experiments with regard to stability and non extractable residues formation are recommended to widen the interpretation field of SPM analysis.

This study shows that SPM analysis is also successful for micropollutants with low K_d, such as carbamazepine (10 L/kg). Positively charged compounds such as amisulpride (log D of -0.08) could be detected in all SPM samples due to a K_d of 119 L/kg, although relatively low average water concentrations with 15 ng/L were detected in Koblenz in 2015 (average of thirteen 28-day composite sample).

In certain cases, compounds could be detected in SPM, but were frequently below LOQ in the water phase. For example, only 5% of sertraline was sorbed onto SPM (value estimated from the K_d), while its concentrations in surface water were seldom above the LOQ of 20 ng/L. On the contrary, in SPM, sertraline could be detected at all sampling locations. To conclude, SPM analysis is particularly relevant for the analysis of positively charged and relatively nonpolar pharmaceuticals such as sertraline or fluoxetine, since these are rarely detected in the water phase.

3.4 Conclusion

- SPM is appropriate to investigate the pollution status of a water systems with micropollutants even for relatively polar compounds. For micropollutants with elevated sorption affinities ($K_d \leq 500$ L/kg) the analysis of SPM is recommended, especially for trend analysis since lower emissions can be detected.

- Sorption of positively charged pharmaceuticals is significant due to the electrostatic interactions even for rather polar compounds such as guanylurea.

- Temporal trends between 2005 and 2015 revealed the ubiquitous presence of pharmaceuticals in SPM of the river Rhine and the river Saar. For many pharmaceuticals, a distinct correlation was observed between the German pharmaceutical consumption and the concentrations detected in SPM.

- The monitoring of composite SPM samples offers the opportunity to get hints about specific industrial discharges by comparing the pollution pattern along a river. This underlines the suitability of SPM analysis as a valuable tool for water monitoring.

Acknowledgements

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4

Development of an analytical method to quantify pharmaceuticals in fish tissues by LC-MS/MS detection and application to environmental samples

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Abstract

A sensitive multiresidue method was developed to quantify 35 pharmaceuticals and 28 metabolites/transformation products (TPs) in fish liver, fish fillet and fish plasma via LC-MS/MS. The method was designed to cover a broad range of substance polarities. This objective was realized by using non-discriminating sample clean-ups including separation technique based on size exclusion, namely restricted access media (RAM) chromatography. This universal clean-up allows for an easy integration of further organic micropollutants into the analytical method. Limits of quantification (LOQ) ranged from 0.05 to 5.5 ng/mL in fish plasma, from 0.1 to 19 ng/g d.w. (dry weight) in fish fillet and from 0.46 to 48 ng/g d.w. in fish liver. The method was applied for the analysis of fillets and livers of breams from the rivers Rhine and Saar, the Teltow Canal as well as carps kept in fish monitoring ponds fed by effluent from municipal wastewater treatment plants. This allowed for the first detection of 17 analytes including 10 metabolites/TPs such as gabapentin lactam and norlidocaine in fish tissues. These results highlight the importance of including metabolites and transformation products of pharmaceuticals in fish monitoring campaigns and further investigating their potential effects.

4.1 Introduction

Pharmaceuticals and their human metabolites are continuously entering the sewage as a result of domestic consumption and inappropriate disposal of unused medicines from hospitals and households [190, 278]. They can reach wastewater treatment plants (WWTP), where most of them are only partly mineralized or removed [99]. From there the remaining pharmaceutical parent compounds, their metabolites and the transformation products (TPs) formed on-site are then discharged into the adjacent water bodies [196]. Increasing consumption of medicinal products [279] and their highly-specific biological activity have raised numerous concerns about their environmental impact and triggered regulatory initiatives, e.g. the European Union Strategic Approach to Pharmaceuticals in the Environment [280]. Metabolites and TPs have to be considered as well, as their toxicity can be comparable or even higher than those of their parent pharmaceuticals as outlined by Escher et al. [173].

In the aquatic environment, fish can be exposed to pharmaceuticals through the gills, dermal absorption from the surrounding water and dietary intake of food and particulate, e.g. suspended particulate matter, macrozoobenthos and prey fish [146]. Some micropollutants are only partly metabolized in fish and may then accumulate in fish tissue or be excreted unchanged [144]. Recent studies have shown that certain pharmaceuticals, in particular selective serotonin reuptake inhibitors (SSRIs) and nonsteroidal anti-inflammatory drugs (NSAIDs) can alter physiological functions of fish at concentrations found in the WWTP effluents and in surface water [281, 282]. Moreover, due to the high number of pharmaceuticals present as mixtures in the aquatic environment, additive or synergistic effects cannot be excluded [171].

Due to the analytical challenges associated with the detection of micropollutants in aquatic biota, the first detection of pharmaceuticals in fish was only performed in 2005, i.e. finding SSRIs in fish from municipal effluent dominated streams [283]. Since then several authors have developed methods for pharmaceutical analysis in fish. However, most of them included only a limited number of analytes (typically less than 35) [284–286]. To date, only Grabicova et al. [287] reported the investigation of an elevated number of pharmaceuticals. The study focused on 64 medicinal parent compounds and 10 metabolites but did not include transformation products (TPs) or pharmaceuticals which were conjugated with biochemical intermediates after phase II metabolism in fish.

In our study, we developed a sensitive analytical method for the quantification of 63 analytes (Table C.1), including 35 pharmaceuticals and 28 metabolites/TPs in fish fillet, fish liver and fish plasma via LC-MS/MS. The analytes were selected due to their environ-

mental relevance as well as to cover a large range of chemical properties (polarity, pKa). The method could easily be extended to include further pharmaceuticals/micropollutants. To the best of our knowledge, more than 30 compounds were investigated for the first time in wildlife fish tissues and 17 of them could be quantified in this study (Table C.2). The method was applied to detect pharmaceuticals and their metabolites and TPs in bream liver, fillet and plasma from the German rivers Rhine, Saar and the Teltow Canal as well as in fillet and liver from carps bred in fish monitoring ponds fed by effluents from municipal WWTPs in Bavaria (Southern Germany).

4.2 Material and methods

4.2.1 Chemicals

LC-MS grade acetonitrile, methanol, tetrahydrofuran (THF), formic acid and acetic acid (all Lichropur®), GC-MS grade ethyl acetate (Suprasolv®), dimethyl sulfoxide (DMSO, Uvasolv®) and β-glucuronidase from *Helix pomatia* (Type H-2, aqueous solution ≥ 100,000 units/mL) were purchased from Merck (Darmstadt, Germany). GC-MS grade acetone and n-heptane (Promochem®) were purchased from LGC (Wesel, Germany). Sodium acetate, LC-MS grade cyclohexane and dichloromethane were purchased from Carl Roth (Karlsruhe, Germany). Ammonia solution (25%) was purchased from AppliChem (Darmstadt, Germany). Milli-Q® (18.2 MΩ.cm, Merck Millipore) was utilized as ultrapure water. The analytical standards and internal standards suppliers are listed in Table C.3 and C.4. Individual stock solutions at 1 g/L were prepared in appropriate solvents. From these solutions, a multi-standard solution was prepared in methanol. Glucuronide standards and their suppliers are listed in Table C.5. Glucuronide standard individual stock solutions at 1 g/L were prepared in DMSO.

4.2.2 Method development

Method development was performed in fish liver as it was assumed to be the most complex matrix. In the following, development and optimization of the different preparation steps are described.

Glucuronide cleavage To evaluate the glucuronide cleavage, six commercially available glucuronide and sulfate metabolites with different bond types (O-glucuronide, acyl glucuronide, N-glucuronide, see Table C.5) were mixed in a 0.01 mg/L solution in DMSO. 50 µL of this mix was spiked in 2 mL sodium acetate buffer at pH 4.7 (adjusted with acetic acid). After adding 20 to 60 µL of 10000 units/mL β-glucuronidase, the samples

were agitated overnight at 37 °C. They were measured by LC-MS to evaluate the cleavage efficiency (glucuronide MRMs reported in Table C.5).

Extraction For extraction, ultrasound extraction (USE), pressurized liquid extraction (PLE) and cell disruption were compared. USE was performed by 2 cycles of 15 min extraction with 5 mL n-heptane followed by 2 cycles of 15 min extraction with 4 mL acetonitrile. PLE was performed with n-heptane (3 cycles, 15 min static time), methanol/Milli-Q (1/1, v/v, 1 cycle, 15 min) and methanol/2%$_v$ formic acid (1/1, v/v, 2 cycles, 15 min) by 100 °C and 100 bar (based on the method from Boulard et al. [288]). Cell disruption was performed with n-heptane and acetonitrile according to the procedure described in 4.2.3.

Clean-up: elimination of fat interferences Diol solid phase extraction (SPE) cartridges (6 mL, 1000 mg, Chromabond, Machery-Nagel) were compared with silica gel SPE (corresponding procedure described in 4.2.3). After loading of the n-heptane extract, the analytes were eluted with 5 × 2 mL dichloromethane, 5 × 2 mL methanol/acetone (4/6, v/v) and 5 × 2 mL methanol, 0.5%$_v$ NH_3. All fractions were collected separately and analyzed by LC-MS.

Clean-up: elimination of protein disturbances Several procedures were compared. Shodex CLNpak PAE-800 AC column (8.0 × 300 mm, 5 µm, Shodex Showa Denko Europe GmbH) was tested with acetonitrile and acetone as eluents. The flow rate was 0.5 mL/min and the injection volume 500 µL. A Phenogel column (7.8 × 300 mm, 5 µm, 50 Å, Phenomenex) was also evaluated with tetrahydrofuran (THF) and THF/acetone (70/30, v/v) as eluents. A flow rate of 1 mL/min and an injection volume of 500 µL were used. In addition, Bio-Beads S-X3 material (Bio-Rad), filled in a GPC column (bed length 320 mm, inner diameter 25 mm) was tested with cyclohexane/ethyl acetate (50/50, v/v) and THF as eluent using a flow rate of 5 mL/min and an injection volume of 4 mL. Finally, Lichrospher® RP-8 ADS (25 × 4 mm, 25 µm, Merck KGaA, Darmstadt) was evaluated according to the procedure described in 4.2.3.

4.2.3 Final sample preparation

The developed analytical method is schematically illustrated in Figure 4.1. In the following, the optimized conditions for the different sample preparation steps are described.

Glucuronide cleavage For plasma samples, 200 µL aliquot was pipetted into a 15 mL polypropylene Falcon tube containing 2 mL of 10 mM sodium acetate buffer (adjusted to

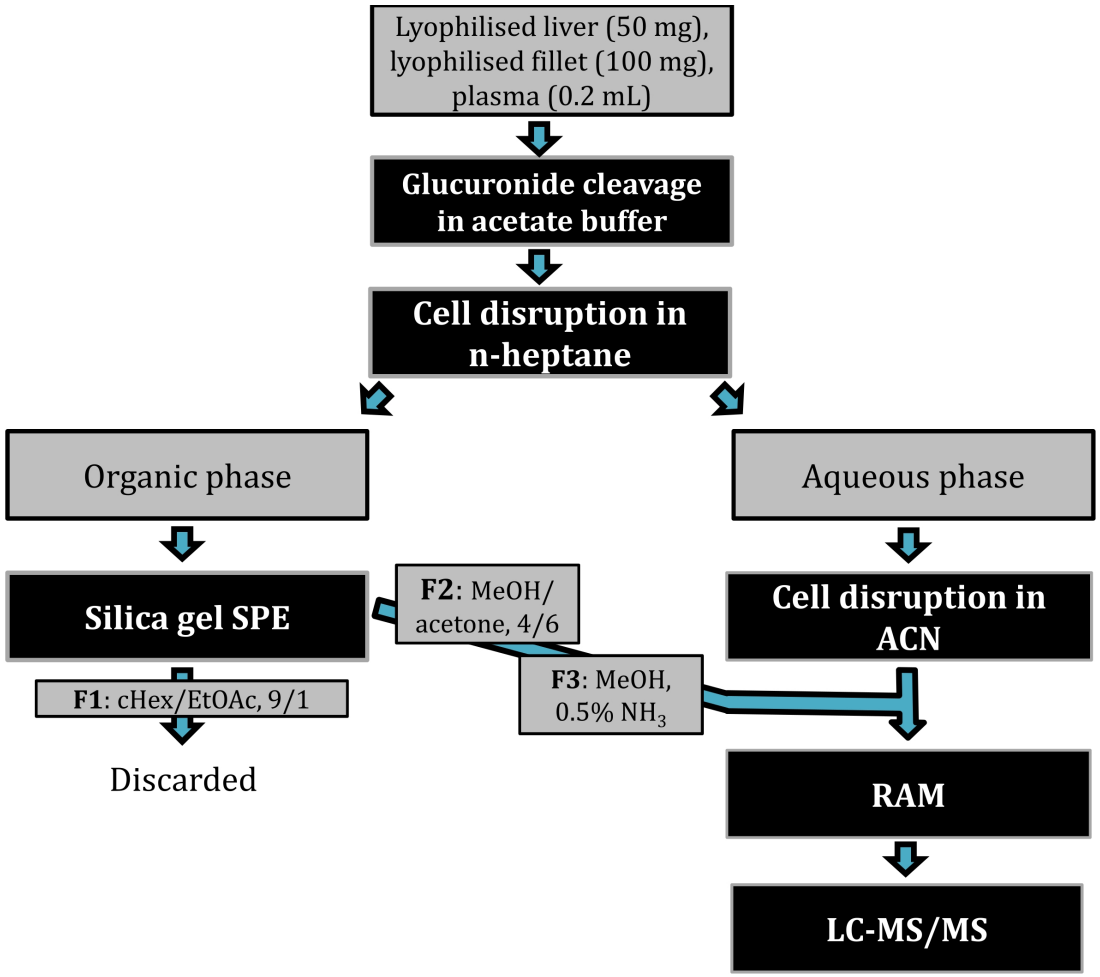

Figure 4.1: Optimized analytical sample preparation method. MeOH: methanol, ACN: acetonitrile, cHex: cyclohexane, EtOAc: ethyl acetate.

pH 4.7 with acetic acid) and 100 µL of 0.01 mg/L internal standard solution were added to the samples.

Homogenized lyophilized fish tissue (50 mg for fish liver or 100 mg for fish fillet) was weighed into a 15 mL polypropylene Falcon tube containing 500 mg of garnet matrix A (MP Biomedicals, Illkirch-Graffenstaden, France) and 150 mg of lysing matrix D (MP Biomedicals). 2 mL of 10 mM sodium acetate buffer (adjusted to pH 4.7 with acetic acid) and 100 µL of 0.01 mg/L internal standard solution were added to the samples. Subsequently, cell disruption was performed in a FastPrep-24^{TM} 5G (MP Biomedicals) equipped with a CoolTeenPrep^{TM} adapter at 4.0 m/s for 40 s.

Afterwards, for all matrices, 20 µL β-glucuronidase (10000 units/mL) was added and the samples were agitated overnight (at least 14 h) at 37 °C and 150 rpm in an orbital incubator SI500 (Stuart, Staffordshire, United Kingdom).

Sample extraction The following day, 5 mL n-heptane was added to the samples and they were extracted by cell disruption at 4 m/s for 40 s in a FastPrep-24^{TM} 5G. Subsequently, the extracts were centrifuged at 6000 rpm (3420 g) for 5 min in a Hettich Mikro 200R (Tuttlingen, Germany). The organic phase was separated, the samples were extracted a second time according to the same procedure and the organic phases were combined. Afterwards, the samples were extracted with 4 mL ice cold acetonitrile by the same cell disruption procedure, centrifuged for 15 min at 6000 rpm and 4 °C before removal of the supernatant. The residues were extracted a second time, centrifuged for 5 min at 6000 rpm and 4 °C and the supernatants were combined. Both extracts (n-heptane and acetonitrile) were then evaporated to 2 mL. To ensure dissolution of all analytes, the n-heptane extracts were vortexed and ultrasonicated for 10 min before silica gel SPE.

Silica gel SPE Silica gel cartridges (6 mL, 1000 mg, Chromabond, Machery-Nagel, Düren, Germany) were dried at 85 °C for 3 hours prior to utilization. They were conditioned with 3 × 2 mL n-heptane before loading the n-heptane extracts. Elution was performed in three steps. The first fraction was eluted with 3 × 2 mL cyclohexane/ethyl acetate (9/1, v/v), the second with 3 × 2 mL methanol/acetone (4/6, v/v) and the third with 3 × 2 mL methanol, 0.5%$_v$ NH_3. To minimize analyte losses, the sample vessel was rinsed with each of the elution solvents. All fractions were collected separately and the second fraction was directly eluted into the vessel containing the acetonitrile extract. The first fraction was discarded. Fractions 2 (already containing the acetonitrile extract) and 3 were evaporated to 1 mL and combined. 10 µL formic acid was added to avoid the sorption of the positively charged analytes, which is particularly critical during solvent exchange to water. The subsequent preparation of the extracts for RAM (restricted access

media) chromatography differed between liver and fillet/plasma samples and is described separately in the following two paragraphs.

Preparation of fillet and plasma samples for the RAM chromatography The extracts were evaporated to 100 µL and 100 µL acetonitrile was added to ensure the re-dissolution of the most nonpolar analytes. The samples were vortexed for 30 s and ultrasonicated for 10 min. Subsequently, 800 µL Milli-Q was added, the samples were vortexed again and centrifuged at 18000 rpm (30800 g). The supernatants were transferred into vials for injection in the RAM.

Preparation of liver samples for the RAM chromatography Due to elevated matrix loads, liver extracts required a further precipitation step for sample clean-up. The extracts were evaporated to dryness and re-dissolved in 100 µL Milli-Q. 900 µL acetonitrile was added to precipitate the impurities. The samples were centrifuged at 18000 rpm (30800 g) for 10 min and 900 µL supernatant was transferred into an evaporation vial. The extracts were evaporated to exactly 100 µL. To ensure the re-dissolution of the most nonpolar substances, 100 µL acetonitrile was added to the samples, they were vortexed for 30 seconds and ultrasonicated for 10 min. Subsequently, 700 µL Milli-Q was added and the samples were centrifuged at 18000 rpm for 10 min. The supernatants were transferred into vials for injection in the RAM.

RAM The RAM was performed with an Agilent 1260 system equipped with a G1364C fraction collector (Agilent, Waldbronn, Germany). A Lichrospher RP-8 ADS (25 × 4 mm, 25 µm) was utilized with 0.1%$_v$ formic acid as eluent A and acetonitrile as eluent B. The flow rate was set to 1 mL/min. The following gradient was applied: 0-3 min: 2% B, 3-3.5 min: 2 to 60% B, 3.5-8.5 min: 60% B, 8.5-9 min: 60 to 98% B, 9-14 min: 98% B, 14-14.5 min: 98 to 2% B, 14.5-20 min: 2% B. The eluate was collected between minute 3 and 13. The injection volume was 500 µL. Subsequently, 10 µL formic acid was added to the collected fractions and they were evaporated to 1 mL before measurement with LC-MS.

LC-MS analysis The LC-MS method is described in detail in Table C.6 and MS-specific parameters are given in Tables C.3 and C.4. Briefly, reversed phase LC-MS/MS was utilized based on the method from Hermes et al. [265].

4.2.4 Quantification

Quantification was based on a 14-point calibration ranging from 0.5 to 5000 ng/L with a linear model and a 1/x weighting factor. If available, stable isotope labeled internal

standards were used to compensate matrix effects (Table C.7). Otherwise, the internal standard with the most similar structure and retention behavior was used instead. Data processing was performed with the software Multiquant^{TM} 3.0.2 (SCIEX, Darmstadt, Germany).

4.2.5 Method validation

Method validation was performed with lyophilized bream liver from Koblenz, Rhine, km 590 and bream fillet from Lake Belau and with pike blood plasma from Lake Stechlin.

Repeatability of the method (intra-day precision) was tested at 10 ng/g and 50 ng/g for fillet and liver and at 2.5 and 10 ng/mL for plasma by the repeated injection of the samples (n=5). Inter-day precision was evaluated at the same concentration levels by the injection of the samples on 4 different days.

The accuracy of the method was verified by determining the recoveries at 3 different concentration levels in the three matrices (fillet, liver and blood plasma). As uncontaminated fish samples were not available, the original analyte concentrations were substracted to calculate the recoveries. Relative recoveries were calculated by normalizing the area of the analytes by that of a corresponding isotope-labeled internal standard (Table C.7). Reproducibility of the method was evaluated by the determination of the 95% confidence intervals between the four replicates of each spike level. The LOQ was defined as the concentration at which a signal-to-noise ratio (S/N) of 10 was reached for the MS/MS transition used for quantification and at least a S/N of 3 for the MRM transition used for confirmation. The LOQ was determined in sample extracts over three separately extracted spiked tissues. Matrix effects were determined by spiking sample extracts after completion of the clean-up and comparing their analyte peak areas with those of a standard at the same concentration.

4.2.6 Environmental samples

For the determination of environmental concentrations, breams were sampled at two sampling locations on the river Rhine: Koblenz, Rhine km 590, Bimmen, km 863 and one location on the river Saar, Rehlingen, km 54. These samples were provided by the German Environmental Specimen Bank (ESB), a permanent monitoring infrastructure managed by the German Environment Agency and financed by the German Environmental Ministry. The sampling procedure is described in detail by Klein et al. [289]. Briefly, sampling was performed after the spawning period (from mid July to the end of October). At least 20 breams (eight to twelve years old) were captured at each sampling location. The breams were dissected and liver and fillet were immediately frozen on site in liquid nitrogen. Fish

livers and fillets of the different individuals were pooled prior to cryo-milling and processing into aliquots. Afterwards, the samples were archived for long-term storage on liquid nitrogen at below -150 °C. According to a similar procedure, eleven breams were sampled from the Teltow Canal in 2016 and individually frozen. Blood plasma samples were stored individually.

In addition, common carp fillets and livers were provided by the Bavarian Environmental Agency, which stores remaining sample material of its monitoring programs for several years. The samples were two year old carps which were kept in monitoring ponds filled exclusively with WWTP effluent water from March to October 2015. The carps were not fed with fish food during this time to prevent any external contamination [290]. The fish were dissected, the tissues were immediately wet homogenized and the samples were frozen at below -20 °C by the Bavarian Environmental Agency.

All samples were shipped on dry ice and placed directly after arrival in the freezer at -80 °C prior to freeze-drying. Lipid content of the tissues are given in Table C.8.

4.2.7 Lipid determination

For fish fillet from Rehlingen and Koblenz, lipid content was obtained from the German ESB [291]. For the other samples, lipid content was determined according to Smedes method.

4.3 Results and discussion

4.3.1 Method development

4.3.1.1 Cleavage of enzymatic conjugates

Glucuronidation and sulfation are the major phase II metabolic reactions in fish [292]. However, it is challenging to directly analyse glucuronide and sulfate conjugates due to the lack of commercially available standards. As an alternative, conjugates can be cleaved enzymatically [293, 294]. The enzyme β-glucuronidase from *Helix pomatia* exhibits both glucuronidase and sulfatase activity.

The procedure was tested with a mixture of six commercially available glucuronide and sulfate metabolites including O-, N- and acyl glucuronide and sulfate (Table C.5). O-glucuronide, acyl glucuronide, sulfate and N2-glucuronide were cleaved quantitatively. However, no cleavage of the N1-glucuronide bond could be observed. According to the literature [293], N-glucuronide can be cleaved only chemically under acidic conditions. However, for our model compound, the diphenhydramine N-glucuronide, only a minor

proportion was cleaved under these conditions (<1%). Alternatively, more reactive cleaving agents such as hydrazine hydrate could have been used but due to a possible side reaction they would have been unsuitable in a method aiming to investigate of a broad range of pharmaceuticals. For example, hydrazine hydrate can reduce carbonyl groups into methylene (Wolff-Kishner reduction) preventing the correct quantification of the analytes with ketone moieties.

4.3.1.2 Optimization of the extraction

Three extraction methods PLE, USE and cell disruption were compared using commonly used conditions as a starting point. For PLE extraction, three 15 min extraction cycles were performed with n-heptane followed by one extraction cycle with methanol/Milli-Q (1/1, v/v) and finally two extraction cycles with methanol/2%$_v$ formic acid (adapted from [288]). For USE extraction, two 15 min extractions were performed with n-heptane followed by two 15 min extractions with acetonitrile. For cell disruption extraction, two 40 s extractions were performed with n-heptane at 4 m/s followed by two 40 s extractions with acetonitrile at 4 m/s. All methods provided similar extraction efficiencies with a comparable reproducibility (Figure C.1). Cell disruption was selected due to its high throughput with a total extraction time of 2.7 min in comparison to 60 min for USE and 90 min for PLE.

Removal of lipids is a critical step in sample preparation of biota. Since certain analytes were quite nonpolar, co-extraction during removal of lipids by solvent extraction was a challenge. For cell disruption, the solvents n-hexane/dichloromethane (1/1, v/v), n-hexane/dichloromethane (3/1, v/v), n-hexane/dichloromethane (9/1, v/v), n-hexane and n-heptane were compared as initial solvents to remove most of the lipids. Surprisingly, all tested solvents co-extracted some of the analytes (Figure 4.2, C.2). In particular, n-hexane/dichloromethane (1/1, v/v) extracted at least partially 42 of the 63 analytes and even with n-heptane or n-hexane nine analytes were partially extracted (Figure 4.3). For example, carbamazepine was extracted to 100% by n-hexane/dichloromethane (1/1, v/v) and already to 20% with n-heptane (Figure 4.3, C.2). We finally selected n-heptane to separate lipids from analytes as efficiently as possible and for safety and environmental considerations. For recovering co-extracted analytes, we included an SPE step using silica gel as an adsorbent (see 4.3.1.3).

Based on reports in the literature [283, 285, 295], extraction by cell disruption should be repeated with acetonitrile for extraction of the more polar analytes. This provided satisfactory extraction recoveries including for the most polar analytes (see 4.3.1.3).

Subsequently, the effect of prolonged extraction times on the recoveries and on the

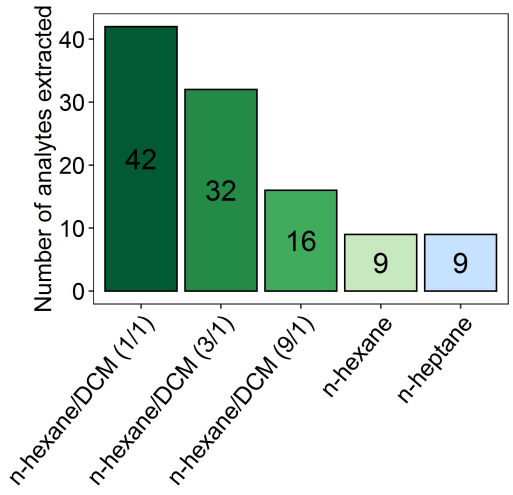

Figure 4.2: Number of analytes co-extracted to more than 10% by the first extraction solvent according to the utilized extraction solvent. DCM: dichloromethane.

extraction of non-spiked residues was evaluated. However, no significant effect could be seen (Figure C.3).

4.3.1.3 Silica gel SPE

After extraction, the n-heptane extract was transferred to silica gel SPE columns to recover co-extracted analytes. In order to eliminate as much nonpolar matrix disturbances as possible the eluent was selected as the most polar eluent not eluting any of the analytes (elution step 1). This condition was fulfilled by the cyclohexane/ethyl acetate mixture (9/1, v/v).

Most analytes could be eluted with a mixture of methanol/acetone (4/6, v/v) (elution step 2) but the elution of analytes containing a positively charged amine/azole moiety was challenging. Neither the utilization of non-activated silica gel nor the elution with a very polar solvent such as methanol/Milli-Q (95/5, v/v) led to sufficient elution. Instead, methanol supplemented with 0.5%$_v$ NH_3 was applied (elution step 3) to deprotonate the amine/azole moieties of the analytes and avoid any electrostatic interaction with the negative surface charge of the silica. Thereby, an almost complete elution was finally achieved. Under these conditions, the neutral clopidogrel was recovered quantitatively from the silica gel SPE (96%) as well as the positively charged sertraline (71%) and the negatively charged diclofenac (99%).

By combining SPE eluates (fraction 2 and 3) and acetonitrile extracts, satisfactory extraction recoveries were obtained for all analytes (Table C.9). For example, sulpiride (log D at pH 7 = -1.07 [296]) showed quantitative extraction recoveries (96 ± 11%) as

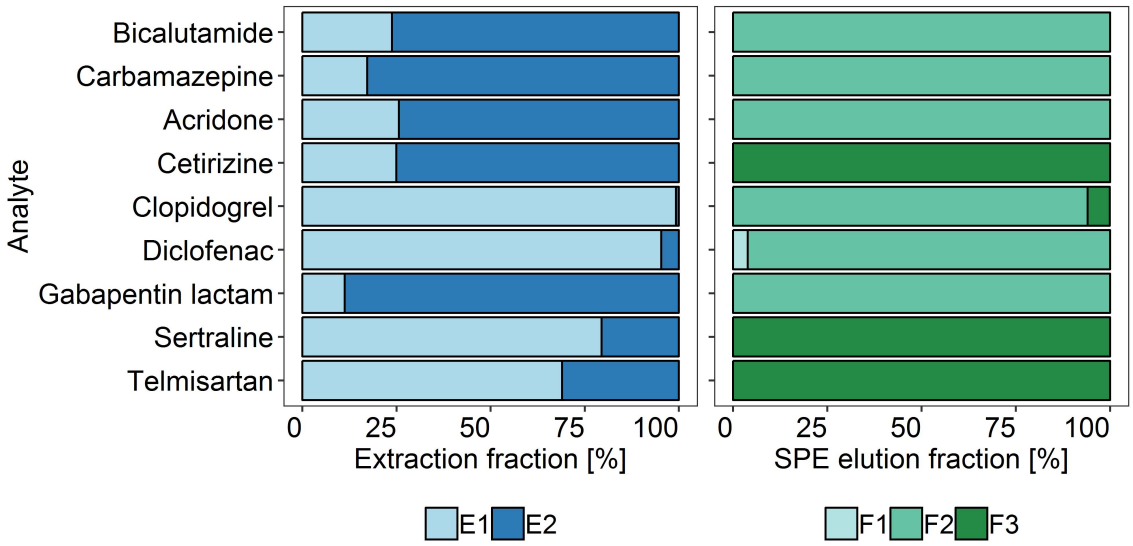

Figure 4.3: Partitioning of the analytes extracted to more than 10% in the nonpolar fraction, between both extraction solvents and between the different SPE fractions. E1: first extraction solvent, i.e. n-heptane, E2: second extraction solvent, i.e. acetonitrile. F1: first SPE fraction eluted with cyclohexane/ethyl acetate (9/1, v/v), F2: second SPE fraction eluted with methanol/acetone (4/6, v/v) and F3: third SPE fraction eluted with methanol, 0.5%$_v$ NH_3

well as the medium polar citalopram (120 ± 5%) or negatively charged bezafibrate (87 ± 26%). Nonpolar analytes such as clopidogrel were co-extracted by n-heptane but still showed quantitative extraction recoveries (79 ± 10%) after silica extraction and combining of the fractions.

4.3.1.4 Clean-up

When re-dissolving the residues of the extracts in water after the silica gel SPE, a cloudy suspension was formed. Due to very fine particles, this suspension could not be separated by centrifugation or filtration. Consequently, further clean-up was necessary.

Methods for clean-up of fish extracts in the framework of the analysis of polar substances are rare in the literature, but gel permeation chromatography (GPC) is a commonly used method for the elimination of high molecular weight matrix interferences. In a first approach, GPC clean-up using a Shodex CLN pak PAE-800 column was tested, based on the method from Löffler et al. [297]. However, this column showed undesired chemical interaction of the polyvinyl alcohol material with the polar analytes causing unsatisfactory elution times and recoveries. An alternative Bio-Beads column did not offer a suitable clean-up due to solubility issues (with cyclohexane/ethyl acetate as an eluent) and due to insufficient swelling (with THF as an eluent). Based on the method of Schlüsener et al. [298], a Phenogel column was tested as the third option but the separation efficiency was insufficient to make a clear cut-off between the matrix disturbances and the analytes.

As an alternative to the polymeric GPC materials, restricted access media (RAM) can

be used for separation of macromolecules from polar analytes [299]. Using a Lichrospher RP-8 ADS column, satisfactory separation of the analytes from the matrix interferences was obtained, except for the analytes combining high polarity and low molar masses such as gabapentin (log D at pH 3: -1.27, M=171.2 g/mol). For all other analytes, acceptable recoveries were obtained for the RAM clean-up with 57 of 63 tested analytes showing quantitative recoveries (i.e. 70-130%, Table C.10). Illustratively, recoveries of 100 ± 10% were obtained for the very polar analyte sulpiride, 90 ± 10% for the positively charged analyte citalopram, 100 ± 10% for the negatively charged analyte bezafibrate and 101 ± 8% for the nonpolar analyte clopidogrel.

4.3.1.5 Other challenges

One challenge during method development was the re-dissolution of the analytes in Milli-Q water during the solvent exchange phases, in particular for the most nonpolar or positively charged analytes. For the latter, losses could be reduced by addition of formic acid prior to solvent exchanges, as this leads to protonation of the silica moieties on the glass vessel walls limiting sorption. For example, the recoveries of citalopram (positively charged, log D at pH 7=1.01) over the whole sample preparation could be improved from 50 ± 6% to 76 ± 6%[1].

Further losses could be reduced by addition of 100 µL acetonitrile, thorough vortexing and 10 min sonication prior to Milli-Q addition. The sonication step was crucial which is illustrated by an increase of acridone recoveries from 26 ± 1 to 56 ± 4%[1].

Even after previously mentioned optimizations, significant ion suppression effects were observed for liver samples. We therefore decided to evaluate the integration of a pre-clean-up prior to the RAM. For this purpose, the samples were evaporated to dryness then re-dissolved in acetonitrile/Milli-Q (90/10, v/v) permitting the precipitation of the co-extracted matrix compounds. The decrease of ion suppression by inclusion of solvent exchange prior to RAM reduced mean matrix effects from -48% without any pre-clean-up to -5% with solvent exchange (Figure C.4, C.5). For fillet samples, this pre-clean-up did not lead to any significant improvement and was thus not included.

A further challenge was the retention time shift of the positively charged analytes in certain liver samples (retarded elution). Utilization of isotope labeled internal standards, comparison of the MRMs of quantification and confirmation and utilization of spiked samples ensured that the specificity of the method was maintained.

[1]Recoveries over the whole sample preparation with compensation of the matrix effects by addition of internal standard before LC-MS measurement

4.3.2 Method validation

The final analytical method allows for simultaneous detection of 63 analytes in fish fillet, liver and plasma. Method validation included the following criteria: accuracy, reproducibility, instrumental precision (repeatability and inter-day precision), sensitivity and matrix effects. To illustrate the universality of the method, for each criterion, the examples of the very polar analyte sulpiride, the medium polar analyte carbamazepine and the nonpolar analyte clopidogrel (Log D at pH 7: -1.07, 2.77 and 4.03, respectively) will be discussed.

Accuracy of the method was evaluated by the calculation of the relative recoveries at three spike levels: 5, 10 and 50 ng/g d.w. for fillet and liver and 1, 2.5 and 10 ng/mL for plasma. To simplify the notation, these spike levels will be named I, II and III hereafter. Recoveries ranged from 70 to 130% except for seven analytes which showed either slightly higher or lower recoveries, mostly due to the unavailability of the corresponding isotopic labeled substances (Figure 4.4 (a), Table C.11).

Reproducibility of the method was evaluated by the determination of the 95% confidence intervals of the four replicates of each spike level. For most analytes, confidence intervals of <25% (Figure 4.4 (b), Table C.11) indicated a good reproducibility of the method. Exemplary, at spike level I, the very polar sulpiride showed recoveries of 77 ± 16% in fillet, 109 ± 9% in liver and 91 ± 2% in plasma, the medium polar carbamazepine recoveries of 91 ± 4% in fillet, 94 ± 6% in liver and 97 ± 2% in plasma and the nonpolar clopidogrel recoveries of 80 ± 11% in fillet, 70 ± 12% in liver and 99 ± 2% in plasma. This demonstrates the adequate accuracy and reproducibility of the method over the whole polarity spectrum.

Instrumental precision of the detection method was determined by the repeated injection of the extracts (spike levels II and III) on the same day (n=5, intra-day precision) and on four different days (n=4, inter-day precision). All analytes exhibited intra-day relative standard deviations (RSD) below 20% and in average even below 4% in fillet, liver and plasma (Table C.12). Inter-day RSD was also below 20% for all analytes except sertraline and the average inter-day precision was below 5% (Table C.12). For example, at spike level II in liver, sulpiride showed an RSD of 1.8% for the intra-day and of 2.9% for the inter-day precision, carbamazepine exhibited RSDs of 1.8% and 1.7%, respectively and clopidogrel of 1.2% and 0.8%.

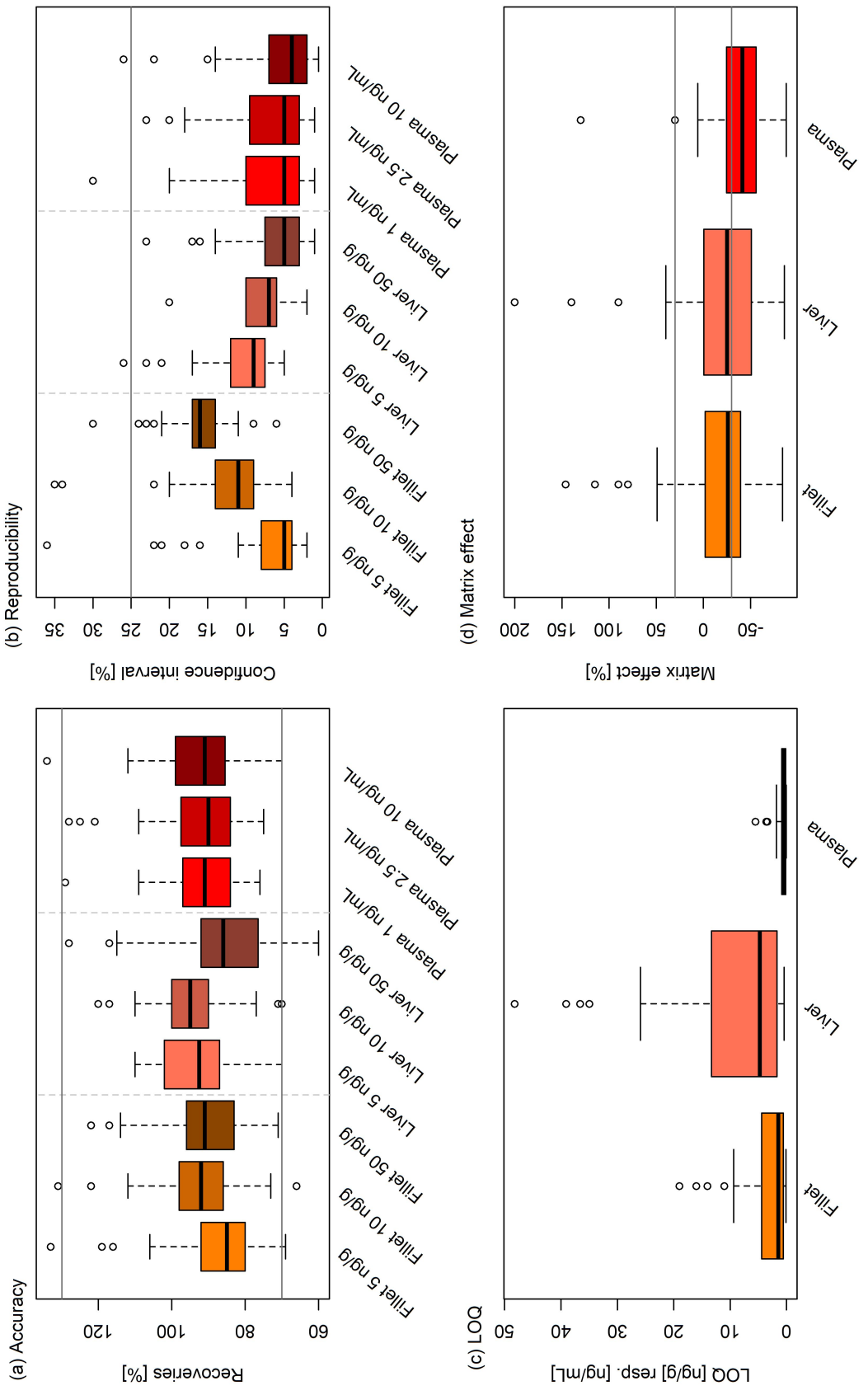

Figure 4.4: Summary of the validation results. (a) Boxplot of the relative recoveries at the different spike levels. (b) Boxplot of the 95% confidence intervals between the four replicates of the same spike level. (c) Boxplot of the limits of quantification. (d) Boxplot of the matrix effects.

LOQs were determined in real sample extracts as the minimal concentration at which a S/N of 10 was attained for the MRM of quantification and S/N of 3 for the MRM of identification. In fillet, LOQs ranged from 0.1 ng/g d.w. (clopidogrel) to 19 ng/g d.w. (O-desmethylmetoprol), in liver from 0.46 ng/g d.w. (bicalutamide) to 48 ng/g d.w. (N-acetylsulfamethaxole) and in plasma from 0.05 ng/mL (bicalutamide) to 5.5 ng/mL (furosemide) (Figure 4.4 (c)). Lower LOQs in fillet than in liver can be mainly attributed to a higher sample mass utilized for fillet compared to liver.

To date, no standard method has been established for the determination of LOQs in fish. Many authors utilized method detection limits (MDL), which is either defined as the standard deviation between seven replicate analyses of a spiked matrix sample multiplied by the one-sided t-Student factor or as the standard deviation between seven replicate extractions of a spiked sample multiplied by the one-sided t-Student factor, as the lower boundaries for quantification. Tanoue et al. [285] compared MDL[2] and LOQ (defined from signal to noise ratio) and showed that derived LOQ were higher by a factor 2 to 9. Moreover, most authors deriving their LOQ by S/N do not apply the LOD criterion (S/N >3) for the MRM transition used for confirmation which might reduce the specificity and reliability of the detections.

LOQ levels were comparable with the literature (Table 4.1). For example, for carbamazepine, a LOQ of 0.73 ng/g d.w. (i.e. 0.15 ng/g w.w.) was determined in fillet. If the calculation of the LOQ were only based on the MRM used for quantification and not on the additional criterion of a S/N >3 for the second MRM transition used for qualification, the LOQ of carbamazepine would be considerably lower (0.22 ng/g d.w., i.e. 0.04 ng/g w.w.). LOQs based only on the criterion of S/N >10 for the quantifier were in the same range or even lower than those obtained by others authors for most analytes for which corresponding literature data was available (Table 4.1). In particular, Grabicova et al. [287], whose multi-residue method scope is similar to ours, determined a LOQ of 0.22-0.38 ng/g w.w. for carbamazepine in fillet, i.e. a factor 5 to 9 higher than our quantifier based LOQ (0.04 ng/g w.w.). However, Arnnok et al. [300] reported lower LOQ than ours, but their analytical method contained only 22 analytes giving them the possibility to apply a more specific clean-up approach. For clopidogrel, we derived in fillet an LOQ of 0.10 ng/g d.w. (i.e. 0.02 ng/g w.w.), this LOQ was the same as that determined by Huerta et al. [301] or Moreno et al. [302] and was a factor eight to eighteen lower than the LOQ determined by Rojo et al. [303]. In liver, clopidogrel LOQ (0.65 ng/g d.w.) was very similar to the one determined by Moreno et al. [302] (0.6 ng/g d.w.) or Huerta et al. [284] (0.87 ng/g d.w.). In total, 13 pharmaceuticals, 17 metabolites and 4 TPs were analyzed

[2]Defined as MDL=2σ(n=7, replicate extraction)t(n-1, 95%)

for the first time in fish tissues and thus no comparison with literature values was possible.

Table 4.1: Comparison of the obtained LOQ with literature data for the analytes. For determined LOQ, numbers in parenthesis correspond to theoretical LOQ only based on the MRM of quantification and without criterion on the MRM of confirmation. Bold numbers corresponds to LOQ in wet weight, numbers in italic to MQL, underlined numbers to MDL and double underlined numbers to LOD.

Analytes	Matrix	Determined LOQ [ng/g] ([ng/mL] for plasma)	Literature LOQ [ng/g] ([ng/mL] for plasma)
Bezafibrate	Fillet	0.49	7.42-18.38[a] **0.41-0.67[b]** **0.033[c]**
	Liver	0.94	**0.069-0.097[b]** **0.27[d]** **0.26[c]**
	Plasma	0.18	0.20-0.41[b] 0.062[d] 0.05[e] 0.0051[c]
Carbamazepine	Fillet	0.73 (0.22)	1.92-1.97[a] 0.3[f] 0.1[g] 0.04[h] *0.035*[i] **0.22-0.38[b]** **1.2[j]** ***0.092-0.18[k]*** **0.012[c]** **0.55[l]** **0.19[m]** **0.54[n]** **0.16[o]**
	Liver	1.64 (0.62)	0.3[f] 0.25[h] *0.065*[i] **0.24-0.33[b]** **0.18[d]** **0.048[c]** **0.94[l]** **0.16[o]**
	Plasma	0.17 (0.04)	0.18-0.36[b] 0.020[d] 0.05[e] 0.3-0.6[p] 0.010[c] 0.16[q]
2-Hydroxycarbamazepine	Fillet	0.45	0.2[f] 0.1[g] 0.26[h] ***0.063-0.25[k]***
	Liver	1.96	0.8[f] 0.83[h]
	Plasma	0.15	n.a.
10,11-Dihydroxy-10,11-dihydrocarbamazepine	Fillet	1.4	**0.43-0.70[b]**
	Liver	10.58	**0.86-1.2[b]**
	Plasma	0.23	n.a.
Cetirizine	Fillet	4.5	**0.13-0.21[b]**
	Liver	16.47	**0.049-0.068[b]**
	Plasma	0.44	0.098-0.20[b]
Citalopram	Fillet	1.2	0.6[f] 0.4[g] 0.16[h] *0.084*[i] **0.24-0.40[b]** **0.062-0.24[r]** **1.4-2.4[s]**
	Liver	4.73	1.7[f] 0.29[h] *0.249*[i] **0.19-0.27[b]** **0.090-0.8[r]** **0.9-2.8[s]**
	Plasma	0.26	0.095-0.19[b] 0.40-1.9[r] 1.4-2.4[s] 0.25[t] 0.08-0.13[p]
Desmethylcitalopram	Fillet	1.2	**0.15-0.25[b]**
	Liver	2.7	**0.23-0.32[b]**
	Plasma	0.38	0.098-0.20[b]
Clopidogrel	Fillet	0.1	0.1[f] 0.1[g] 0.26[h] ***0.16-0.37[k]***
	Liver	0.65	0.6[f] 0.87[h]
	Plasma	0.073	n.a.
Diclofenac	Fillet	2.4	79.14-303.36[a] 0.4[f] 0.2[g] 0.62[h] *0.345*[i] **0.20-0.33[b]** **0.03[j]** ***6.9-9.1[k]*** 0.35[u] **0.042[c]** **2.31[o]**
	Liver	2.52	0.4[f] 2.16[h] *1.12*[i] **8.4-12[b]** **0.83[d]** **0.21[c]** **2.31[o]**
	Plasma	0.41	0.68-1.4[b] 0.15[d] 0.1[e] 1[y] 0.012[c] 2.31[q]
Diphenhydramine	Fillet	0.29	*0.048*[i] **0.2[j]** **0.025[c]** **0.07[l]** **0.04[m]** **0.05[n]** **0.11[o]**
	Liver	0.67	*0.130*[i] **0.12[d]** **0.028[c]** **6.0[l]** **0.11[o]**
	Plasma	0.22	0.028[d] 0.12-0.24[p] 0.010[c] 0.13[q]
Fexofenadine	Fillet	1.6	**0.034-0.053[b]**
	Liver	14.2	**0.28-0.39[b]**
	Plasma	0.28	0.25-0.5[b] 0.5[e]
Fluconazole	Fillet	0.43	**0.13[w]**
	Liver	1.32	**0.61[w]**
	Plasma	0.21	n.a.
Fluoxetine	Fillet	4.7 (2.59)	**0.3-3.4[s]** **0.07[x]** **0.05[y]** **5.3[l]** **0.78[m]** **6.73[n]** **0.36[o]**
	Liver	7.49 (6.95)	**1.0-6.4[s]** **0.05[y]** **5.7[l]** **0.36[o]**
	Plasma	0.47 (0.24)	1[e] 0.3-3.4[s] 0.25[t] 0.3-0.6[p] 0.85[q]

Analytes	Matrix	Determined LOQ [ng/g] ([ng/mL] for plasma)	Literature LOQ [ng/g] ([ng/mL] for plasma)
Norfluoxetine	Fillet	5.5	*0.040*[i]; **0.14**[x] **0.05**[y] **3.0**[l]; **0.83**[m] **2.9**[n] **0.71**[o]
	Liver	34.94	*0.122*[i]; **0.05**[y] **6.7**[l]; **0.71**[o]
	Plasma	0.83	2.50[t]; 0.365-0.6[p]; 0.99[q]
Furosemide	Fillet	3.2	n.a.
	Liver	17.75	n.a.
	Plasma	5.5	8-16[p]
Hydrochlorothiazide	Fillet	1.3	0.05[f]; 0.2[g] 0.57[h]; ***0.21-0.239***[k]
	Liver	3.31	0.2[f]; 0.35[h]
	Plasma	0.33	4-4.8[p]
Metoprolol	Fillet	3	23.76-26.19[a]; 0.6[f]; 0.7[g] 0.60[h]; **0.17-0.23**[b] ***0.14-0.60***[k]; **2.5**[n]
	Liver	9.34	2.0[f]; 1.19[h]; **0.028-0.041**[b]
	Plasma	1.3	0.055-0.10[b]; 0.3-0.6[p]
Oxazepam	Fillet	1.3	**0.19-0.33**[b] **0.5**[z]
	Liver	3.28	**0.76-1.1**[b]
	Plasma	0.48	0.20-0.45[b]; 0.5[e]; 0.8-1.33[p]
Primidone	Fillet	2	0.06[u]
	Liver	7.35	n.a.
	Plasma	0.58	n.a.
Sertraline	Fillet	9.4	2.6[f]; 1.1[g]; 0.61[h]; *0.092*[i]; **0.094-0.15**[b]; **0.32-1.2**[r]; **1.3-5.6**[s]; **0.05**[y]; **0.017**[c]; **2.1**[l]; **0.53**[m]; **3.57**[n]; **0.99**[o]
	Liver	9.58	22.1[f]; 3.28[h]; *0.182*[i]; **0.075-0.11**[b]; **0.48**[d]; **0.020-0.18**[r]; **1.0-5.2**[s]; **0.05**[y]; **0.11**[c]; **9.6**[l]; **0.99**[o]
	Plasma	0.91	0.22-0.45[b]; 0.19[d]; 0.5[e]; 0.5-0.86[r]; 1.3-5.6[s]; 0.25[t]; 0.08-0.16[p]; 0.022[c]; 0.99[q]
Sulfamethoxazole	Fillet	4.8	*0.662*[i]; **0.13-0.20**[b] **0.03**[j]; 0.05[u]; **2.9**[l]; **0.72**[m] **2.29**[n]; **1.87**[o]
	Liver	16.84	*4.51*[i]; **0.33-0.61**[b]; **3.8**[l]; **1.87**[o]
	Plasma	1.1	0.24-0.47[b]; 1[y]; 0.12-0.24[p]; 1.9[q]
N-Acetyl sulfamethoxazole	Fillet	14	*4.46*[i]; **0.062-0.11**[b]
	Liver	48.17	*21.4*[i]; **11-20**[b]
	Plasma	1.6	0.79-1.5[b]
Telmisartan	Fillet	0.59	**0.14-0.23**[b]
	Liver	0.96	**0.28-0.39**[b]
	Plasma	0.086	0.23-0.46[b]; 1[e]
Tramadol	Fillet	0.49	**0.71-1.2**[b]; **0.085-0.14**[r]
	Liver	1.39	**0.33-0.54**[b]; **0.78-2.5**[r]
	Plasma	0.16	0.11-0.22[b]; 0.1[e]; 0.40-0.64[r]
Trimethoprim	Fillet	0.82	9.41-28.30[a]; *0.162*[i]; **0.090-0.14**[b]; **0.03**[j]; **3.5**[l]; **1.09**[m]; **2.15**[n]; **0.45**[o]
	Liver	2.01	*0.434*[i]; **0.090-0.15**[b]; **2.3**[l]; **0.45**[o]
	Plasma	0.16	0.058-0.11[b]; 1[y]; 0.3-0.6[p]; 2.8[q]
Valsartan	Fillet	5.3	**0.13-0.22**[b]
	Liver	36.6	**1.3-1.8**[b]
	Plasma	1.7	0.80-1.6[b]; 0.8-1.6[p]
Venlafaxine	Fillet	2.4 (0.97)	1.04-1.82[a]; 0.09[f]; 0.1[g]; 0.55[h]; *0.260*[i]; **0.16-0.27**[b]; **0.40-1.5**[r]; **1.3-2.9**[s]; *0.27-1.25*[k]
	Liver	1.68 (0.94)	0.5[f]; 1.33[h]; *1.10*[i]; **0.18-0.26**[b]; **0.32-1.1**[r]; **1.0-3.4**[s]

Analytes	Matrix	Determined LOQ [ng/g] ([ng/mL] for plasma)	Literature LOQ [ng/g] ([ng/mL] for plasma)
	Plasma	0.76 (0.37)	0.10-0.19[b]; 0.34-1.7[r]; 1.3-2.9[s]; 0.25[t]; 0.13-0.16[p]
O-Desmethyl venlafaxine	Fillet	5.65 (1.1)	*0.249*[i]; **0.18-0.25[b]**
	Liver	12.39 (4.63)	*1.50*[i]; **0.15-0.23[b]**
	Plasma	1.0 (0.27)	0.083-0.15[b]

[a] [304] [b] [287] [c] [305] [d] [285] [e] [306] [f] [302] [g] [301] [h] [284] [i] [300] [j] [286] [k] [303] [l] [307] [m] [308] [n] [309] [o] [310] [p] [311] [q] [312] [r] [313] [s] [314] [t] [315] [u] [316] [v] [317] [w] [318] [x] [319] [y] [283] [z] [282]

Matrix effects varied from -84% (hydrochlorothiazide) to 146% (telmisartan) in fish fillet, from -86% (phenytoin) to 200% (telmisartan) in fish liver and from -88% (hydrochlorothiazide) to 130% (telmisartan) in fish plasma (Figure 4.4 (d), Table C.13). Average matrix effects of -18% and -17% were determined in liver and fillet, respectively. In fish plasma, matrix effects were slightly stronger with an average of -36%. However, 29 analytes in fillet, 26 analytes in liver and 19 analytes in plasma showed low matrix effects between -30% and +30%. Moreover, the matrix effects could be successfully compensated for by the use of isotopic labeled internal standards, as shown by the acceptable accuracy (mostly between 70% and 130%, Figure 4.4 (a), Table C.11) and reproducibility (95% confidence intervals mainly below 25%, Figure 4.4 (b), Table C.11).

4.3.3 Application to environmental samples

To get an overview about the occurrence of pharmaceuticals in fish in freshwater environments, the analytical method was applied to liver, fillet and plasma of breams from the rivers Saar and Rhine, fillet and liver of bream from the Teltow Canal as well as fillets and livers of carps from fish monitoring ponds fed by effluents of five different WWTPs. Selected chromatograms are shown in Figure C.6.

4.3.3.1 Comparison of the different sampling sites

Fish samples were collected from riverine sites with different levels of treated wastewater contribution. The river Rhine, the river Saar and the Teltow Canal represent surface waters with low (5-7% [288]), medium (15% [288]) and high wastewater (up to 100% in the summer) contributions, respectively. Common breams were analyzed due to their widespread occurrence in aquatic ecosystems. Additionally, carps kept for eight months in WWTP fish monitoring ponds were analyzed reflecting an exposure to close to 100% treated wastewater. These carps showed the highest detection frequency for the analyzed

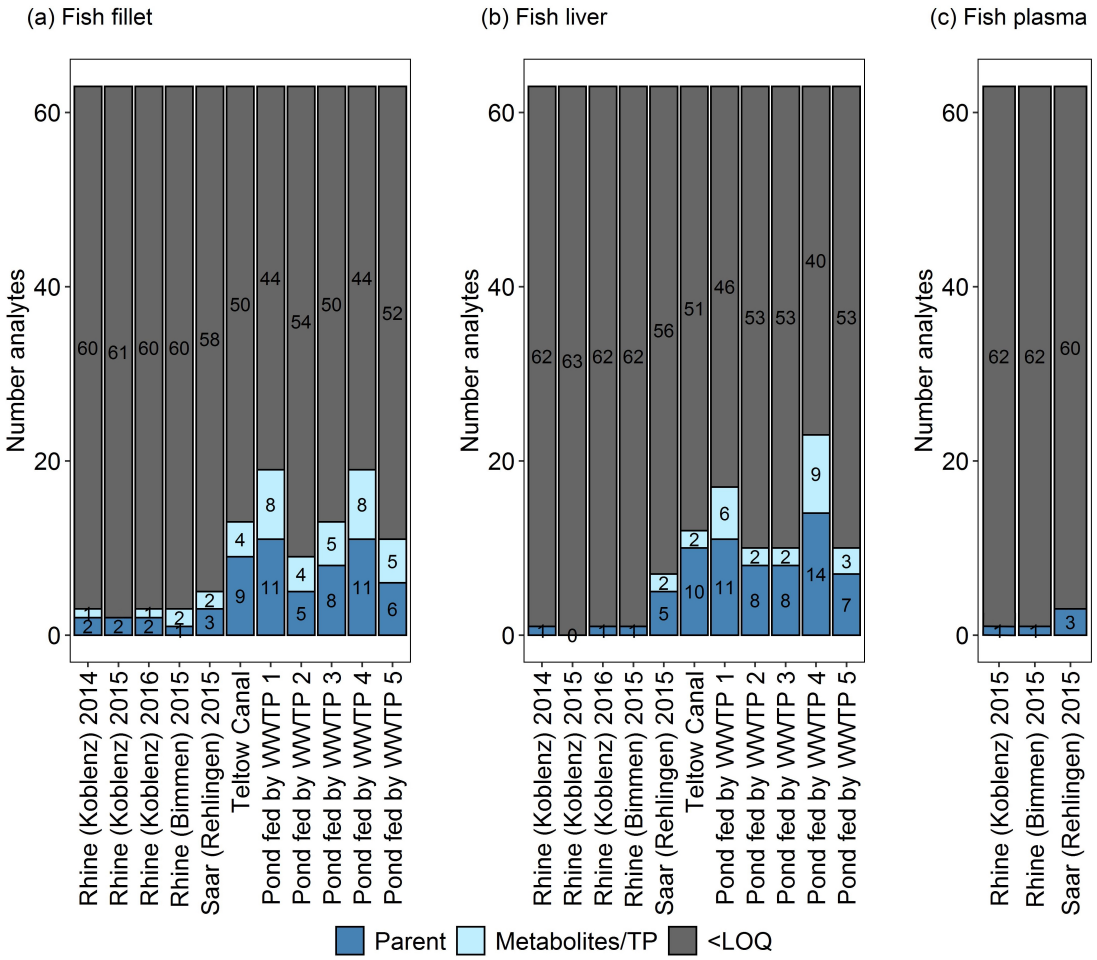

Figure 4.5: Overview of the number of parent pharmaceuticals and corresponding metabolites/TPs detected at quantifiable levels at the different sampling locations. For the Teltow Canal, the example of sample U8 was taken.

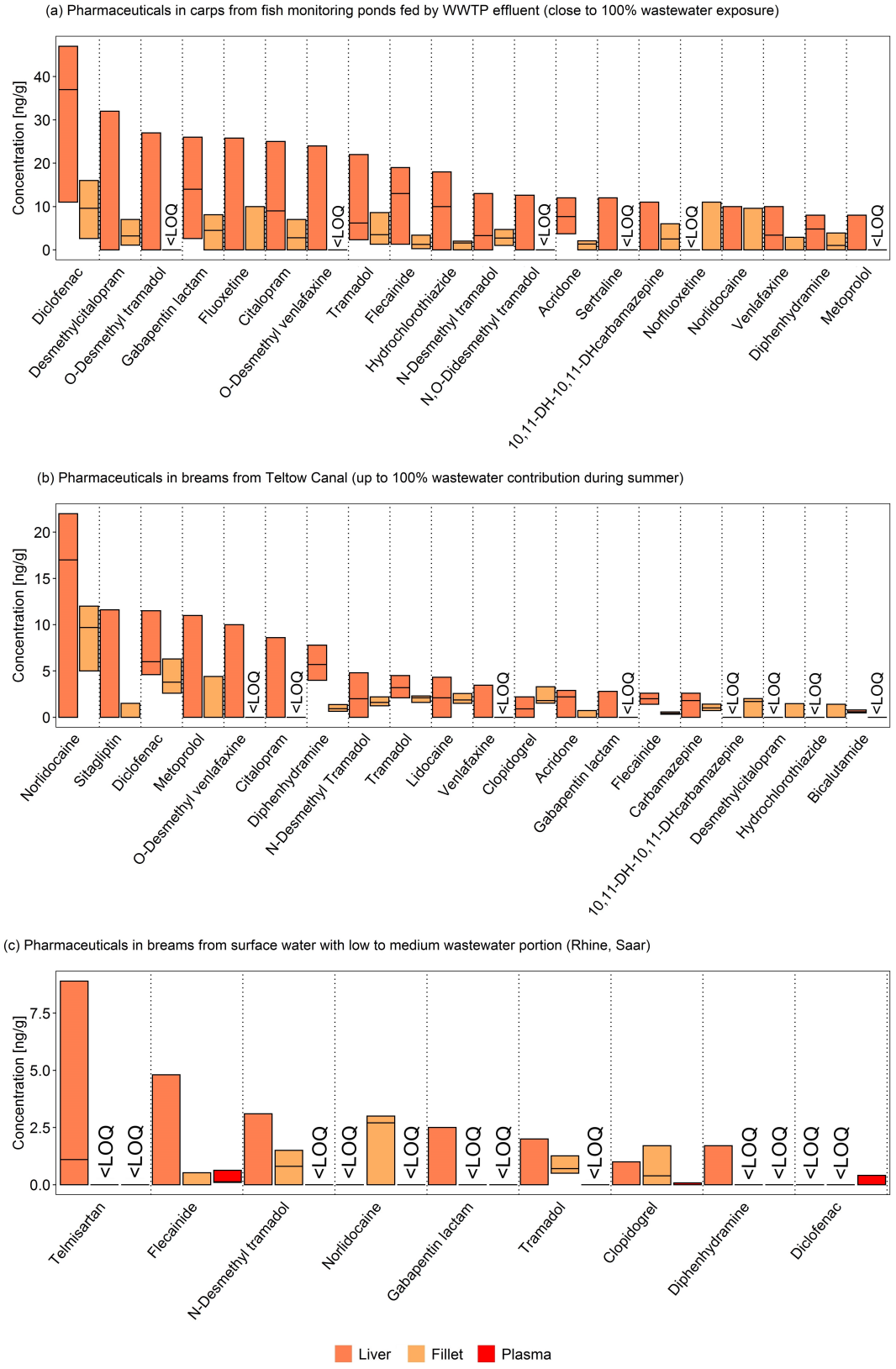

Figure 4.6: Minimal, median and maximal concentrations of pharmaceuticals in (a) carps from WWTP effluents fish monitoring ponds. Due to the high number of analytes detected only the 20 analytes with the highest concentrations are shown, (b) breams from the Teltow Canal, (c) breams from surface water with low wastewater portion (Rhine, Saar). Concentrations in ng/g dry weight for liver and fillet tissues and in ng/mL for plasma. 10,11-DH-10,11-DHcarbamazepine: 10,11-Dihydroxy-10,11-dihydrocarbamazepine.

pharmaceuticals and metabolites/TPs (Figure 4.5). In total, 32 of 63 analytes were detected in at least one fish sample indicating the uptake of most of the selected analytes by carps (Table C.14). In breams of the Teltow Canal up to 13 analytes were detected in one sample and 20 analytes could be detected at least once (Figure 4.6, Table C.15, C.16), all of which were also detected in the carps growing in WWTP fish monitoring ponds at similar concentration levels. This is consistent with the elevated wastewater proportion of the Teltow Canal, which can reach levels up to 100% from April to October. Nine pharmaceuticals were detected at least once in bream samples from the river Saar and Rhine (Figure 4.6, Table C.17). All of them were detected in breams caught in the Saar at Rehlingen (Table C.17). Altogether, concentrations were lower in the Saar and Rhine compared to the other sampling sites. Concentration patterns of the analytes differ strongly between the sampling sites. While diclofenac and desmethylcitalopram showed the highest concentrations of all compounds in carps from WWTP fish monitoring ponds, these analytes were detected in medium concentrations in breams from the Teltow Canal and not detectable in breams from the Rhine or the Saar. Conversely, norlidocaine and sitagliptin showed the highest concentrations in breams from the Teltow Canal but played a minor role in the contamination of carps from WWTP fish monitoring ponds. This is quite surprising, since discharge patterns via wastewater are expected to be comparable between the different sampling sites. The differences in fish concentrations might be caused by species specific uptakes or metabolization rates. In summary, there is a general positive relationship between the wastewater contamination of the water body and the detected residues in fish (Figure 4.5, 4.6).

4.3.3.2 Comparison of different tissues

To investigate the distribution of the analytes within the fish; liver, fillet and blood plasma were separately analyzed. In general, highest concentrations were measured in liver followed by fillet and then plasma, while clopidogrel was found in slightly higher concentrations in fillet (Table 4.2, C.14-C.17). In one carp sample, lidocaine and norlidocaine showed similar concentrations in fillet and liver (Table 4.2). For certain analytes, a correlation could be observed between concentrations in liver and fillet ($p < 0.05$) over all sites (Table C.18, Figure C.7). No relationship was determined between polarity of the analytes and their liver to fillet concentration ratios (Table C.19, $p = 0.75$, Figure C.8). In plasma, only three analytes were detected at very low concentration levels: clopidogrel, diclofenac and flecainide. Only flecainide showed similar concentrations in plasma and in fillet, but concentrations measured in liver were still higher (Table 4.2). Thus, due to the challenge to collect sufficient amounts of plasma material for chemical analysis, plasma seems to be

a rather inconvenient matrix for monitoring pharmaceuticals in fish.

The correlation between analyte concentrations and lipid content of fillet samples was evaluated for the 11 individual bream samples from the Teltow Canal due to their similarity concerning exposure, age and environmental conditions. For all detected analytes the p-values of the correlation were >0.01 and mostly >0.05, thus as expected there is no correlation with the lipid content (Table C.20, Figure C.9), whereas it has been shown for breams that for nonpolar contaminants such as hexachlorobenzene, polychlorinated biphenyls and polybrominated biphenylethers, the lipid content plays a crucial role in accumulation [320].

Table 4.2: Fillet, liver and blood plasma average concentrations and 95% confidence intervals for selected analytes

	Carp Pond fed by WWTP effluent 1 (n=3)		Bream Teltow Canal U2 (n=3)		Bream Rehlingen 2015 (Saar) (n=3)		
Analytes	Fillet ng/g	Liver ng/g	Fillet ng/g	Liver ng/g	Fillet ng/g	Liver ng/g	Plasma ng/mL
Clopidogrel	1.0 ± 0.1	< 0.65	2.8 ± 0.2	1.2 ± 0.4	1.7 ± 0.1	1.0 ± 0.2	0.08 ± 0.01
Diclofenac	12 ± 2	47 ± 7	6.2 ± 0.8	10.3 ± 0.1	<1.48	<2.52	0.41 ± 0.08
Diphenhydramine	3.9 ± 0.7	5.8 ± 0.4	1.12 ± 0.08	7.8 ± 0.5	< 0.29	<0.67	<0.22
Flecainide	3.4 ± 0.3	18 ± 1	0.50 ± 0.02	2.6 ± 0.6	0.52 ± 0.06	4.8 ± 0.7	0.63 ± 0.08
Lidocaine	3.9 ± 0.1	3.4 ± 0.3	2.5 ± 0.1	3.6 ± 0.2	<1.30	<1.69	<0.19
Norlidocaine	9.6 ± 0.5	10 ± 3	8.8 ± 0.8	15.3 ± 0.9	2.7 ± 0.7	<6.74	<0.90
Tramadol	8.6 ± 0.7	15 ± 2	2.3 ± 0.2	4.5 ± 0.3	1.26 ± 0.08	2.0 ± 0.8	<0.16

4.3.3.3 Comparison of metabolites and parent compounds

In addition to the parent pharmaceuticals, several metabolites and transformation products were detected in the fish tissues. In the liver as well as in the fillet, concentrations of the metabolites were comparable or even slightly higher than those of their parent pharmaceuticals (Figure 4.6).

In particular, N-desmethyl tramadol, a human metabolite of the analgesic tramadol, showed concentrations in the same range as tramadol (Figure 4.6, Table C.14-C.17). Illustratively, in Teltow Canal samples, N-desmethyl tramadol concentrations ranged from 1.24 ± 0.08 to 2.2 ± 0.4 in fillet and from <2.37 to 4.8 ± 0.6 ng/g d.w. in liver whereas tramadol concentrations ranged from 1.6 ± 0.2 to 2.3 ± 0.2 ng/g d.w. in fillet and from 2.1 ± 0.5 to 4.5 ± 0.3 ng/g d.w. in liver. Norlidocaine, a human metabolite of the local anesthetic lidocaine, was detected in Teltow Canal fish with concentrations of a factor 2 to 9 higher than lidocaine (Figure 4.6, Table C.15, C.16). In fillet of breams from Rhine and Saar, norlidocaine was found at concentrations up to 3.0 ± 0.9 ng/g d.w., while its parent pharmaceutical lidocaine was not detected at all.

Both norlidocaine and N-desmethyltramadol were frequently detected at higher concentrations than their parent pharmaceuticals in fish tissues, whereas the reverse trend

was observed in the water phase [265]. One reasonable explanation is that the metabolites norlidocaine and N-desmethyl tramadol are also formed by the metabolism of their parent pharmaceuticals in fish. This hypothesis is supported by the variations of concentration ratio of metabolites and parent compounds in particular between individual bream samples from the Teltow Canal. For example, fillet concentration ratios for norlidocaine/lidocaine varied between 2.1 and 7.9 for the eleven Teltow Canal breams. The formation of N-desmethyl tramadol in fish was already confirmed in a recent study [321]. The same study also confirmed the metabolization of fluoxetine to norfluoxetine in fish.

Determined concentrations were consistent with previous European studies [284, 287, 308, 313] (Table C.2). Illustratively, we quantified carbamazepine from <1.64 to 2.3 ± 0.3 ng/g d.w. (<0.67 to 0.94 ± 0.01 ng/g w.w.) in livers of carps from fish monitoring ponds fed by Bavarian WWTP effluents, whereas in carps from a Czech Republic treated wastewater dominated pond average concentrations of 0.95 ± 0.29 ng/g w.w. were determined in liver. The same authors did not detect carbamazepine in the respective fillet samples (<0.22-0.38 ng/g w.w.) due to higher LOQ, while we concurrently determined concentration levels ranging from <0.73 to 0.86 ± 0.06 ng/g d.w. (<0.15 to 0.17 ± 0.01 ng/g w.w.).

In total, 17 analytes were detected in fish tissue for the first time in this study (Table C.2, laboratory bioaccumulation experiments not taken into account). In particular, 10 metabolites/TPs could be detected for the first time: three metabolites and TPs of carbamazepine, a metabolite of citalopram, a metabolite of diphenhydramine, a metabolite of lidocaine, three metabolites of tramadol and a TP of gabapentin. Seven parent pharmaceuticals were also detected for the first time: amisulpride, bicalutamide, chlorothiazide, flecainide, lidocaine, quetiapine and sitagliptin. In particular, flecainide, gabapentin lactam, norlidocaine and N-desmethyl tramadol were identified in fish from surface water with low portions of treated wastewater and thus are particularly relevant for further investigations.

Flecainide is a rather polar (log D at pH 7: 0.66) and positively charged antiarrhythmic agent. It was detected in fish fillet from Rehlingen at 0.52 ± 0.06 ng/g d.w., in liver at 4.8 ± 0.7 ng/g d.w. and in fish plasma at 0.63 ± 0.08 ng/mL. In the Teltow Canal, its concentrations ranged from 0.32 ± 0.07 to 0.58 ± 0.08 ng/g d.w. in fillet and from 1.4 ± 0.3 to 2.6 ± 0.6 ng/g d.w. in liver. Flecainide was also detected in all carp samples from fish monitoring ponds fed by WWTPs at concentrations between 0.29 ± 0.06 and 3.4 ± 0.3 ng/g d.w. in fillet and between 1.3 ± 0.6 and 19 ± 7 ng/g d.w. in fish liver.

Gabapentin lactam, a TP of the antiepileptic drug gabapentin [322], was also detected for the first time in fish. Concentration of 2.5 ± 0.4 ng/g d.w. was quantified in fish liver

Rehlingen sampled in 2015, while it was not detected in bream liver from Koblenz (2015, 2016) and Bimmen (2015). In the Teltow Canal concentrations from <1.51 to 2.8 ± 0.3 ng/g d.w. were determined in liver. Gabapentin lactam could be detected in all WWTP fish monitoring pond carp samples with a maximum concentration of 26 ± 2 ng/g d.w. in liver and 8.1 ± 0.4 ng/g d.w. in fillet. Gabapentin lactam has been reported to be more stable than gabapentin with regard to biotic as well as abiotic degradation [322].

4.4 Conclusions

A sensitive multi-residue method has been developed for the analysis of 35 pharmaceuticals and 28 metabolites/transformation products in fish fillet, liver and plasma. The design of the sample extraction and clean-up enabled the analysis of compounds with a broad range of polarity. Due to the use of non-discriminating clean-up techniques such as size exclusion, additional pharmaceuticals/micropollutants can be easily integrated into the analytical method. Even an adaption for a non-target screening might be feasible.

This study reveals that even polar micropollutants such as pharmaceuticals and their human metabolites are ubiquitously present in fish from German rivers. Detection frequency and concentrations depended on the wastewater proportion at the sampling sites, which is consistent with the knowledge of WWTPs as the main source of pharmaceuticals and personal care products. However, pharmaceuticals were also detected in fish from rivers with low contributions of treated wastewater such as the river Rhine.

For most substances, highest concentrations were found in liver. In plasma a few analytes were found but only rarely and in small concentrations. Due to collection difficulties and low available amount, blood plasma seems to be a rather inconvenient matrix for monitoring pharmaceuticals in fish.

For certain substances such as tramadol or lidocaine, the human metabolites showed higher concentrations than the original pharmaceuticals. To which extent these metabolites can be formed by the metabolism in the fish needs to be further elucidated. However, human metabolites need to be considered in monitoring campaigns to achieve a comprehensive overview about the presence of pharmaceuticals in fish. Furthermore, it needs to be elucidated whether the substances are accumulated from the water phase or via magnification. The present data indicate that the bioaccumulation of pharmaceuticals in fish is considerably lower than for lipophilic compounds. Nevertheless, further research is warranted on the long-term effects of pharmaceuticals in fish populations and the accumulation of pharmaceuticals in fish eating predators.

Acknowledgments

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5

Final conclusions

This study aimed to evaluate the presence of pharmaceuticals, their metabolites and transformation products (TPs) in the different environmental compartments as well as to determine the optimal strategy to investigate pharmaceuticals with different physico-chemical properties in aquatic environmental compartments.

This goal was attained through the development of three high sensitive multi-residue methods, designed to close the current gaps of knowledge concerning the occurrence of pharmaceuticals in the aquatic environment.

First, an analytical method was established for the determination of extreme polar pharmaceuticals, pharmaceutical metabolites and TPs in aqueous environmental matrices. The suitability of hydrophilic interaction liquid chromatography (HILIC) for this purpose was confirmed. However, extreme ion enhancement effects were observed. They could be attributed to the co-elution of the analytes with nitrate or chloride and were successfully compensated by the utilization of a corresponding stable isotope-labeled internal standard. Prolonged equilibration time and low chromatographic robustness of HILIC with regard to variations of the acetonitrile to water ratio were also noticed. In consequence, a thorough sample and eluent preparation have to be performed to ensure the chromatography reproducibility and the lab personal has to be trained specially concerning HILIC specificities. Detection of extreme polar pharmaceuticals, pharmaceutical metabolites and TPs at concentration levels up to the high µg/L range in wastewater treatment plant (WWTP) effluent and up to low µg/L range in rivers, streams and groundwater confirmed their ubiquitous occurrence in the urban water cycle and underlines the importance to include extreme polar pharmaceuticals in monitoring studies.

Subsequently, a multi-residue method was developed to investigate a broad spectrum of pharmaceuticals, pharmaceutical metabolites and TPs in suspended particulate matter (SPM). This comprehensive and high throughput method permits the detection of the analytes down to the high pg/g range. Examination of distribution coefficients between the water phase and SPM showed a high variation in sorption ability among pharmaceuticals. The highest distribution coefficients K_d were measured for nonpolar and positively charged analytes with a strong influence of the positive charge. Investigation of SPM annual composite samples revealed appreciable pharmaceutical concentrations ranging up to the high ng/g range. This was especially the case for positively charged analytes including the very polar metformin and guanylurea. For many pharmaceuticals, a distinct correlation of SPM concentrations with consumption volumes was observed and relationships could be established between consumption and SPM concentrations. Studies of the spatial distribution of the analytes led to the suspicion of industrial discharge for four

pharmaceuticals in the Rhine.

SPM was demonstrated to be a suitable new matrix to find and monitor the concentrations of pharmaceuticals, pharmaceutical metabolites and TPs in the water phase despite more time-consuming analytical methods and low concentrations of SPM in surface water. SPM permits integrative sampling and better conservation of the samples and is thus adequate for the determination of pharmaceutical annual loads. Moreover, in combination with the Environmental Specimen Bank, it enables the retrospective investigation of contamination over decades. In particular, SPM was shown to be a relevant matrix for the investigation of positively charged molecules, which is the case of many pharmaceuticals at environmental pH. For pharmaceuticals with elevated sorption affinity (typically K_d superior to 500 L/kg) lower emissions can be detected in SPM.

Finally, a multi-residue method was established for the determination of a large range of pharmaceuticals, pharmaceutical metabolites and TPs in fish liver, fillet and plasma. The developed method allows for the determination of the analytes at concentrations down to the low ng/g range in fish fillet and liver and down to the high pg/mL range in fish plasma. Investigation of environmental samples revealed that pharmaceuticals are ubiquitously present in fish tissues from German rivers and indicated that they are probably further metabolized by the fish. Detected analytes included notably very polar pharmaceuticals such as the analgesic tramadol. However, fish tissues are inappropriate for water monitoring for several reasons. First, fish analysis is extremely time-consuming due to the matrix complexity. Moreover, the dependability of fish diet to their environment and variable fish metabolization rates limits the comparability of the measurements. Finally, bioaccumulation of pharmaceuticals, pharmaceutical metabolites and TPs is of minor relevance in comparison to other contaminants such as polychlorinated biphenyls (PCBs) or brominated flame retardants due to their elevated polarities. In consequence, detection rates are significantly lower in fish than in SPM or in the water phase.

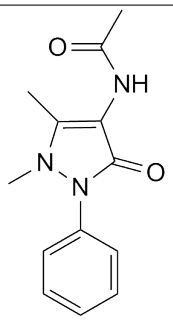
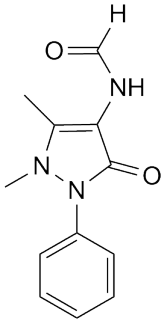
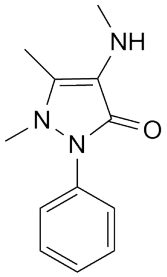
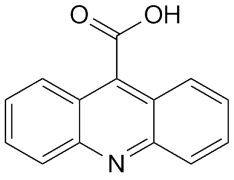
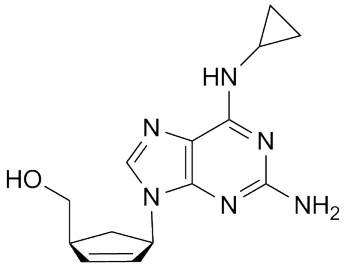
Metabolites and TPs are of high relevance considering the fate and occurrence of pharmaceutical residues in the aquatic environment. In the water phase, polar metabolites could be detected up to the µg/L concentrations in WWTP effluent and in surface water. In SPM, metabolite concentrations were mostly lower than those of their parents, but provide relevant information concerning discharge pathways. In fish tissues, the inclusion of human metabolites in monitoring is fundamental since they may be formed by fish metabolization. TPs were also detected in all three matrices with particularly high concentrations in the water phase.

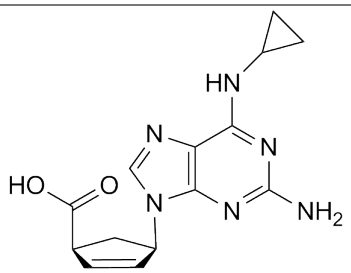
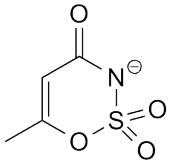
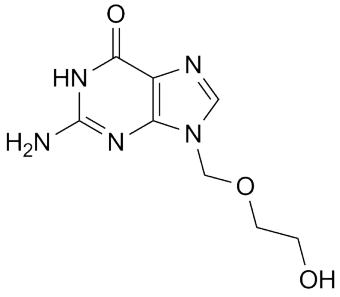
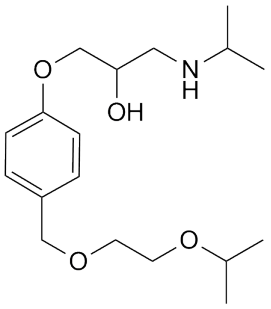
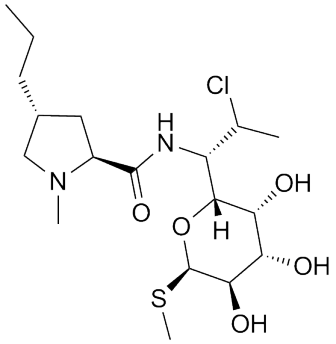
To conclude, the following recommendations can be made concerning the analysis of pharmaceuticals with different physico-chemical properties in the environment. First, in spite of the need to develop specific detection methods, extreme polar pharmaceuticals should be included in the monitoring. For this purpose, HILIC constitutes a suitable detection method providing that the specificities of this analytical technique are considered. Relevant pharmaceutical metabolites and TPs should also be covered in the analytical methods and it is advisable to determine them in all matrices. Moreover, for a comprehensive monitoring, both the water phase and SPM have to be measured. Neutral or negatively charged medium polar pharmaceuticals can be detected in the water phase, where even lower emissions can be covered. For nonpolar and positively charged medium polar pharmaceuticals, determination in SPM is recommended in addition to the water phase. This matrix is also suitable for retrospective analysis. Due to high time-consuming analytical methods, low comparability of the measurements and low detection levels, fish analysis is only recommended for toxicologically relevant pharmaceuticals such as diclofenac.

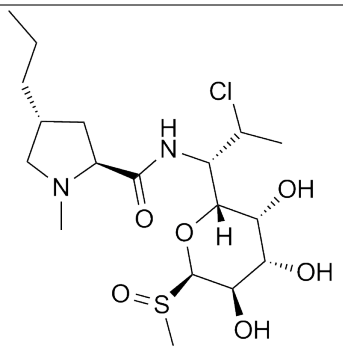
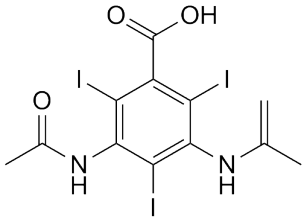
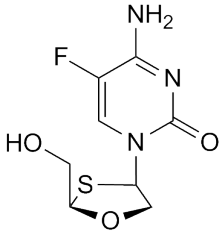
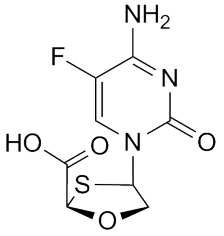
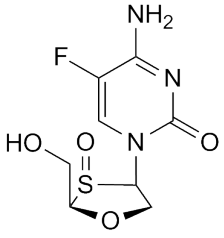
A

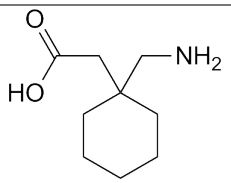
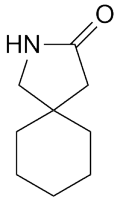
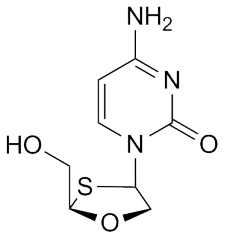
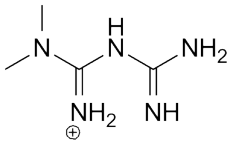
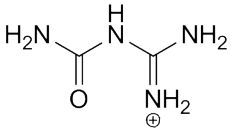
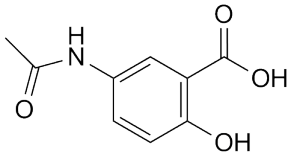
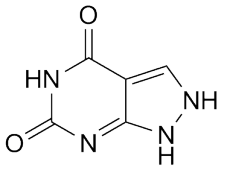
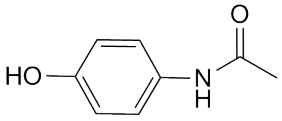
Supplementary data for Chapter 2

Table A.1: Structure of the analytes.

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	

Name	CAS No	Structure
Abacavir carboxylate	384380-52-3	
Acesulfame	55589-62-3	
Acyclovir	59277-89-3	
Bisoprolol	66722-44-9	
Clindamycin	18323-44-9	

Name	CAS No	Structure
Clindamycin sulfoxide	22431-46-5	
Diatrizoate	737-31-5	
Emtricitabine	143491-57-0	
Emtricitabine carboxylate	1238210-10-0	
Emtricitabine *S*-oxide	152128-77-3	

Name	CAS No	Structure
Gabapentin	60142-96-3	
Gabapentin lactam	64744-50-9	
Lamivudine	134678-17-4	
Metformin	657-24-9	
Guanylurea	141-83-3	
N-acetyl mesalazine	51-59-2	
Oxipurinol	2465-59-0	
Paracetamol	103-90-2	

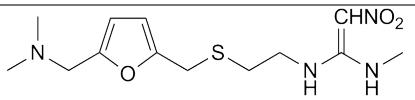
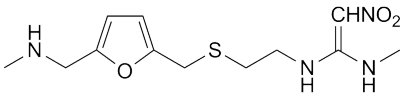
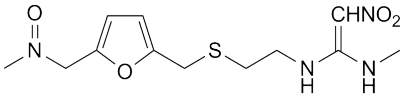
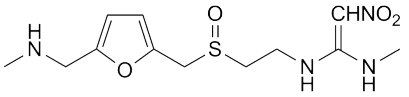
Name	CAS No	Structure
Ranitidine	66357-35-5	
Desmethyl ranitidine	66357-25-3	
Ranitidine *N*-oxide	73857-20-2	
Ranitidine *S*-oxide	73851-70-4	

Table A.2: Multiple reaction monitoring parameters of the analytes.

Name	Retention time [min]	MRM 1 (Quantification)	MRM 2 (Confirmation)	DP [V]	CE [eV]	CXP [V]	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	12.50	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	7.40	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
Guanylurea	14.20	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

Name	Retention time [min]	MRM 1 (Quantification)	MRM 2 (Confirmation)	DP [V]	CE [eV]	CXP [V]	Polarity
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	12.50	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
Guanylurea-$^{15}N_4$	14.20	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

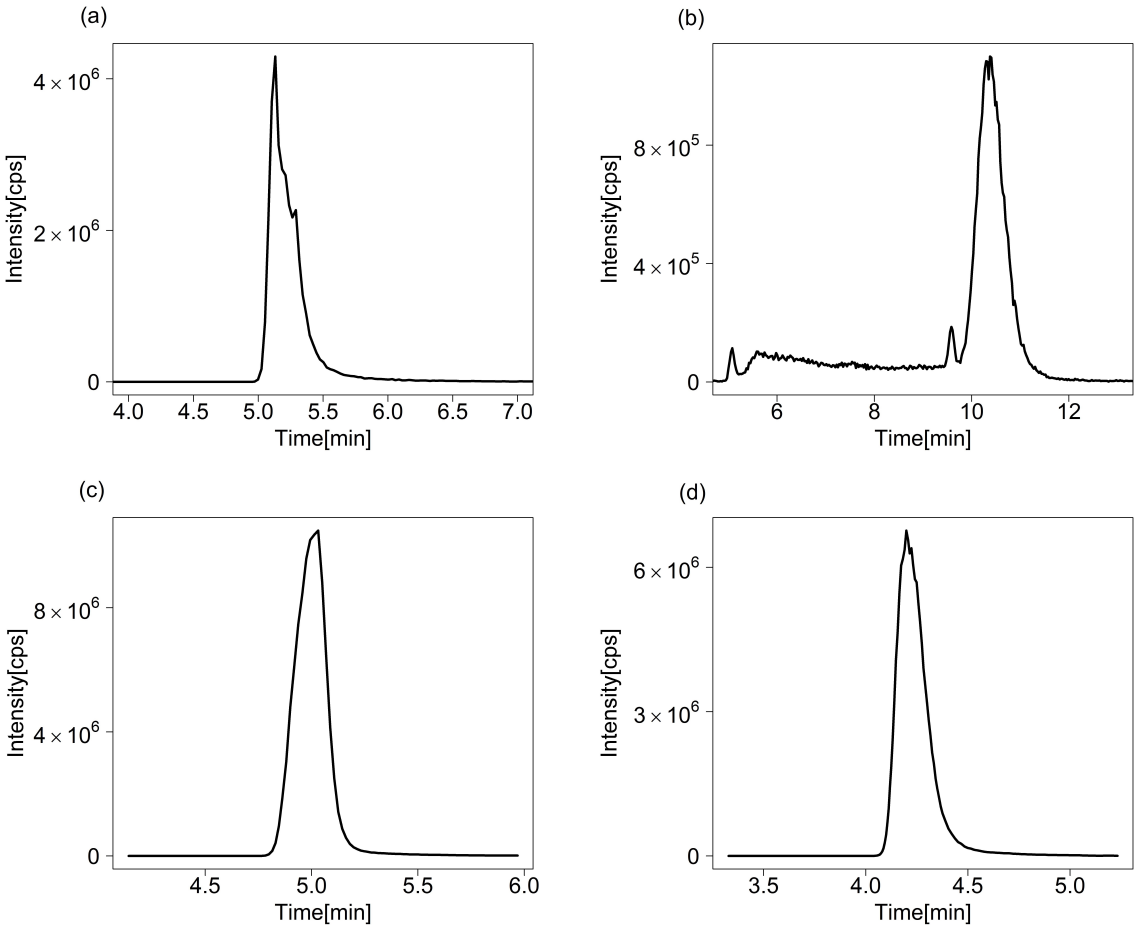


Figure A.1: Comparison of the peak form of 4-acetamidoantipyrine and 4-methylaminoantipyrine with and without ammonium formiate in B. (a) 4-acetamidoantipyrine without ammonium formiate in eluent B. (b) 4-methylaminoantipyrine without ammonium formiate in eluent B. (c) 4-acetamidoantipyrine with ammonium 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.3: 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.
Guanylurea	Guanylurea-$^{15}N_4$
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.

Table A.4: 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
Guanylurea	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.5: Recoveries of the analytes with the different investigated sample preparation procedures

	Recoveries [%]																
Analytes	Oasis MCX pH 2	Oasis MCX pH 3	Oasis MCX pH 5	Oasis HLB pH 2	Oasis HLB pH 3	Oasis HLB pH 5	Isolute ENV + pH 5	Isolute ENV + pH 8	Oasis WCX pH 5.5	Oasis WCX pH 7	Strata XCW pH 5.5	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
Guanylurea	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.6: Calibration information

Analytes	R^2	Range [ng/L]
4-Acetamidoantipyrine	0.99856	0.5-10000
4-Formylaminoantipyrine	0.99820	5-10000
4-Methylaminoantipyrine	0.99898	1-10000
9-Acridine carboxylic acid	0.99894	10-20000
Abacavir	0.99930	5-20000
Abacavir carboxylate	0.99634	20-10000
Acesulfame	0.99788	5-200000
Acyclovir	0.99600	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	0.99710	200-20000
Gabapentin	0.99556	200-100000
Gabapentin lactam	0.99860	5-20000
Lamivudine	0.99898	5-10000
Metformin	0.99906	50-10000
Guanylurea (low calibration)	0.99106	100-10000
Guanylurea (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.7: Multiple reaction monitoring parameters of chloride, nitrate and the sodium adduct of emtricitabine

Name	MRM	DP [V]	CE [eV]	CXP [V]	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.8: 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	Width 5% [min]			Tailing factor		
			peak form with increasing	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	-	0.3	0.31	0.32	1.1	1.1	1.1
Acyclovir	10.81	Slight	ACN	0.34	0.31	0.3	1.0	1.0	1.0
Bisoprolol	8.96	Medium	ACN	0.44	0.37	0.35	0.9	1.1	1
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	-	0.3	0.3	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	0.3	0.3	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	1.1	1.1
Metformin	13.34	Not affected	-	0.31	0.31	0.32	1.1	1.1	1.2
Guanylurea	14.20	Not affected	-	0.24	0.24	0.24	1	1	1
N-Acetyl mesalazine	6.77	Slight	ACN	1.06	1.01	0.97	1.6	2	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	13.22	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.9: Results from the analysis of environmental samples. Concentration in µg/L.

Analyte	WWTP 1 (28/11/2016)	WWTP 1 (6-7/10/2016)	WWTP 1 (11-12/10/2016)	WWTP 1 (15-16/10/2016)	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-Acetamidoantipyrine	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-Formylaminoantipyrine	11.0 ± 0.1	7.6	9	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-Methylaminoantipyrine	<0.02	<0.02	<0.02	<0.02	<0.02	0.055	0.04	<0.02	<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	0.1	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	<0.05	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	0.2	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	0.3	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	0.3	<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	1.10 ± 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 ± 0.001	0.041	0.031	0.04	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
Metformin	4.20 ± 0.01	1.4	0.71	1.1	0.94	0.9	0.89	1.8	0.97 ± 0.03	1.02 ± 0.01	1.10 ± 0.01	1.04 ± 0.07	1.07 ± 0.02
Guanylurea	76 ± 2	4.3	3.8	3.6	110	110	110	100	2.6 ± 0.1	2.70 ± 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	0.3	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	0.0092	<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	0.02	0.021	0.02	<0.005	<0.005	0.0086 ± 0.0002	0.0085 ± 0.0001	0.0085 ± 0.0001

Analyte	Rhine (km 592) 26/03-02/04/17	Rhine (km 590) 26/03-02/04/17	Rhine (km 590) December 2016	Rhine (km 482) 25/03/17-01/04/17	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°; E 8.524°)	Usa 2 (26/07/16, N 50.380°; 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	0.140 ± 0.005	0.171 ± 0.002	0.120 ± 0.006	<0.001	0.17	0.16	0.13	0.011	0.9	0.85	0.82
4-Formylaminoantipyrine	0.081 ± 0.005	0.13 ± 0.01	0.214 ± 0.002	0.100 ± 0.004	<0.002	0.25	0.22	0.12	<0.002	1	0.99	4
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	0.0040 ± 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	0.0060 ± 0.0005	<0.001	0.0033	0.02	0.011	<0.001	0.064	0.037	0.2
Clindamycin	0.014 ± 0.001	0.005 ± 0.0004	0.0117 ± 0.0003	0.0030 ± 0.0001	<0.0005	0.0074	0.018	0.017	<0.0005	0.034	0.026	0.1
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	0.89	1.8
Emtricitabine	<0.001	<0.001	<0.001	<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	0.30 ± 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	0.4	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
Guanylurea	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	<0.2	0.78 ± 0.04	<0.2	<0.2	<0.2	5	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	0.0006	0.01	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	<0.005	<0.005	<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

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 Koblenz-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	0.04	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	0.01	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	0.01	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	0.3	<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	<0.05
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
Guanylurea	<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

B

Supplementary data for chapter 3: Spatial distribution and temporal trends of pharmaceuticals sorbed to suspended particulate matter of German rivers

Table B.1: List of selected analytes, application and log D at pH 7

Name	Cas No	Application	Charge at pH 7[a]	Log D at pH 7[a]
RPLC				
Aliskiren	173334-57-1	Renin inhibitors	Positive	0.680
Amisulpride	71675-85-9	Antipsychotics	Majoritary positive (minoritary form: neutral)	-0.076
Atenolol	29122-68-7	Beta-blocker	Positive	-2.140
Atenolol acid	56392-14-4	TP of atenolol [323], major human metabolite [324] and major TP of metoprolol [119]	Zwitterion	-1.240
Hydroxyatenolol	68373-10-4	Metabolite of atenolol [325]	Positive	-2.855
Bezafibrate	41859-67-0	Lipid regulating agent	Negative	0.973
3-[(4-chlorobenzoyl) amino]propanoic acid	108462-95-9	Ozonation product [326]	Negative	-1.707
Bicalutamide	90357-06-5	Antiandrogen	Neutral	2.709
Candesartan	139481-59-7	Angiotensin	Negative	1.039
Carbamazepine	298-46-4	Antiepileptic	Neutral	2.766
2-Hydroxycarbamazepine	68011-66-5	Metabolite of carbamazepine [327]	Negative	1.370
3-Hydroxycarbamazepine	68011-67-6	Metabolite of carbamazepine [327]	Negative	1.353
10,11-Dihydro-10-hydroxycarbamazepine	29331-92-8	Metabolite of carbamazepine [277]	Neutral	1.732
10,11-Dihydroxy-10,11-dihydrocarbamazepine	29331-92-8	Main human metabolite of carbamazepine [217])	Neutral	0.813
Acridone	578-95-0	Metabolite of carbamazepine, TP of 10,11-dihydro-10-hydroxycarbamazepine and 10,11-dihydroxy-10,11-dihydrocarbamazepine [277]	Neutral	4.199
9-Acridine carboxylic acid	5336-90-3	TP of 10-hydroxy-10-hydroxycarbamazepine and 10,11-dihydroxy-10,11-dihydrocarbamazepine [277]	Majoritary negative (minoritary form: zwitterion)	0.866
Cetirizine	83881-51-0	Antihistamine	Majoritary zwitterion (minoritary form: negative)	0.772
Chlorothiazide	58-94-6	Diuretic	Neutral	-0.445
Citalopram	59729-33-8	Antidepressant	Positive	1.055
Desmethylcitalopram	62498-67-3	Metabolite of citalopram [328]	Positive	0.317
Didemethylcitalopram	62498-69-5	Metabolite of citalopram [328]	Positive	0.140
Clarithromycin	81103-11-9	Antibiotic	Majoritary positive (minoritary form: neutral)	1.840
Clopidogrel	113665-84-2	Cardiac agent	Neutral	4.028
Clopidogrel carboxylic acid	144457-28-3	Main human metabolite of clopidogrel [329]	Majoritary negative (minoritary form: zwitterion)	1.355

Name	Cas No	Application	Charge at pH 7[a]	Log D at pH 7[a]
Diclofenac	15307-86-5	Nonsteroidal anti-inflammatory drug	Negative	1.368
4'-Hydroxy-diclofenac	64118-84-9	Metabolite of diclofenac and primary TP [330]	Negative	0.885
Diclofenac carboxylic acid	13625-57-5	Primary TP of diclofenac [330]	Negative	2.540
Diclofenac lactam	15362-40-0	Primary TP of diclofenac [330]	Neutral	3.802
Diphenhydramine	58-73-1	Antihistamine	Positive	1.790
N-Desmethyl diphenhydramine	53499-40-4	Main human metabolite [331] and anareobic TP [133]	Positive	0.691
Duloxetine	116539-59-4	Antidepressant	Positive	1.605
Fexofenadine	83799-24-0	Antihistamine	Zwitterion	2.940
Flecainide	54143-55-4	Antiarrhythmic agent	Positive	0.663
Flecainide-meta-O-dealkylated	83526-33-4	Main metabolite of flecainide [60]	Positive	-0.391
Fluconazole	86386-73-4	Antifungal	Neutral	0.561
Fluoxetine	54910-89-3	Antidepressant	Positive	1.504
Norfluoxetine	56161-73-0	Main metabolite of fluoxetine [62]	Positive	1.160
Furosemide	54-31-9	Cardiac agent/diuretic	Negative	-0.930
Gabapentin	60142-96-3	Antiepileptic	Zwitterion	-1.273
Gabapentin lactam	64744-50-9	TP of gabapentin [322]	Neutral	1.033
Hydrochlorothiazide	58-93-5	Diuretic agent	Neutral	-0.579
Irbesartan	138402-11-6	Angiotensin	Majoritary negative (minoritary form: neutral)	4.460
Lamotrigine	84057-84-1	Antiepileptic	Majoritary neutral (minoritary form: positive)	1.894
Levetiracetam	102767-28-2	Antiepileptic	Neutral	-0.594
Levetiracetam acid	102849-49-0	Major human metabolite of leveritacetam [69]	Negative	-2.676
Lidocaine	137-58-6	Local anesthetic	Majoritary positive (minoritary form: neutral)	2.019
Nor lidocaine	7729-94-4	Metabolite of lidocaine [71]	Majoritary positive (minoritary form: neutral)	0.520
Metoprolol	37350-58-6	Beta-blocker	Positive	0.807
Hydroxy metoprolol	56392-16-6	Metabolite of metoprolol and TP of metoprolol [119]	Positive	-1.726
O-Desmethyl metoprolol	62572-94-5	Metabolite of metoprolol and TP of metoprolol [119]	Positive	-1.450
Naproxen	22204-53-1	Anti-inflammatory	Negative	0.251
O-Desmethyl naproxen	52079-10-4	Metabolite of naproxen [75]	Negative	0.230
Olmesartan	144689-63-4	Angiotensin	Majoritary negative (minoritary form: zwitterion)	-0.748
Oxazepam	604-75-1	Benzodiazepine	Neutral	2.923

Name	Cas No	Application	Charge at pH 7[a]	Log D at pH 7[a]
Phenytoin	57-41-0	Anticonvulsants	Majoritary neutral (minoritary form: negative)	2.134
Pregabalin	148553-50-8	Antiepileptic	Zwitterionic	-1.346
Primidone	125-33-7	Anticonvulsants	Neutral	1.118
Quetiapine	111974-69-7	Antipsychotics	Majoritary neutral (minoritary form: positive)	2.474
7-Hydroxy-quetiapine	139079-39-3	Metabolite of quetiapine [332]	Majoritary positive (minoritary form: neutral)	2.149
Quetiapine sulfoxide	329216-63-9	Major human metabolite of quetiapine [332]	Majoritary neutral (minoritary form: positive)	1.222
Ritalinic acid	19395-41-6	Main metabolite of methylphenidate [137]	Zwitterion	-0.363
Roxithromycin	80214-83-1	Macrolide antibiotic	Positive	0.931
Sertraline	79617-96-2	Antidepressant	Positive	2.668
N-Desmethyl sertraline	87857-41-8	Major human metabolite of sertraline [333]	Positive	2.314
Sertraline ketone	124379-29-9	Metabolite of sertraline [333]	Neutral	4.910
Sildenafil	139755-83-2	PDE5 inhibitor	Majoritary neutral (minoritary form: negative)	1.229
Sitagliptin	486460-32-6	Antidiabetic drug	Majoritary positive (minoritary form: neutral)	-0.510
Sotalol	3930-20-9	Beta-blocker	Positive	-2.470
Sulfamethoxazole	723-46-6	Antibiotic	Majoritary negative (minoritary form: neutral)	0.140
N-Acetyl sulfamethoxazole	21312-10-7	Metabolite of sulfamethaxole [334]	Majoritary negative (minoritary form: neutral)	0.104
Sulpiride	23672-07-3	Antipsychotic	Majoritary positive (minoritary form: neutral)	-1.074
Tadalafil	171596-29-5	PDE5 inhibitor	Neutral	1.639
Telmisartan	144701-48-4	Angiotensin	Majoritary negative (minoritary form: zwitterion)	5.167
Torasemide	56211-40-6	Diuretic	Majoritary negative (minoritary form: neutral)	1.220
Hydroxytorasemide	99300-68-2	Metabolite of torasemide [89]	Majoritary negative (minoritary form: neutral)	-0.057
Tramadol	36282-47-0	Analgesic	Positive	0.239
Dehydrotramadol	192384-41-1	Metabolite of tramadol [91]	Positive	0.640
O-Desmethyl tramadol	73986-53-5	Metabolite of tramadol [91]	Positive	0.103
N,O-Didesmethyl tramadol	138853-73-3	Metabolite of tramadol [91]	Positive	-0.743
Trimethoprim	738-70-5	Antibiotic	Majoritary positive (minoritary form: neutral)	0.920
3-Desmethyl trimethoprim	27653-69-6	Metabolite of trimethoprim [93]	Majoritary positive (minoritary form: neutral)	0.772
5-(3,4,5-Trimethoxybenzoyl)-2,4-pyrimidinediamine	30806-86-1	TP of trimethoprim [330]	Majoritary neutral (minoritary form: positive)	1.230
Valsartan	137862-53-4	Angiotensin	Negative	1.646

Name	Cas No	Application	Charge at pH 7[a]	Log D at pH 7[a]
Valeryl-4-hydroxyvalsartan	188259-69-0	Metabolite of valsartan [94]	Negative	0.122
Valsartanic acid	164265-78-5	TP of Valsartan [335]	Negative	-0.727
Venlafaxine	93413-69-5	Antidepressant	Positive	0.836
N-Desmethyl venlafaxine	149289-30-5	Metabolite of venlafaxine [95]	Positive	-0.296
O-Desmethyl venlafaxine	93413-62-8	Metabolite of venlafaxine [95]	Positive	0.687
N,O-Desmethyl venlafaxine	135308-74-6	Metabolite of venlafaxine [95]	Positive	-0.431
Xipamide	14293-44-8	Diuretic	Negative	1.190
HILIC				
4-Acetylaminoantipyrine	83-15-8	Metabolite of metamizole [336]	Neutral	0.154
Abacavir	136470-78-5	Antiviral	Neutral	0.361
Acesulfame	55589-62-3	Artificial sweetener	Negative	-1.494
Aciclovir	59277-89-3	Antiviral	Neutral	-1.032
Bisoprolol	66722-44-9	Beta-blocker	Positive	-0.369
Clindamycin	18323-44-9	Antibiotic	Positive	0.379
Clindamycin sulfoxide	22431-46-5	Metabolite of clindamycin [337]	Majoritary neutral (minoritary form: positive)	-1.211
Emtricitabine	143491-57-0	Antiviral	Neutral	-0.896
Lamivudine	134678-17-4	Antiviral	Neutral	-1.095
Metformin	657-24-9	Antidiabetic drug	Positive	-5.692
Guanylurea	141-83-3	TP of metformin [220]	Positive	-3.930

[a] https://chemicalize.com/

Table B.2: Analytes already analyzed in SPM with the corresponding references

Analytes	References
Aliskiren	-
Amisulpride	-
Atenolol	[261], [338], [253], [260]
Atenolol acid	-
Hydroxyatenolol	-
Bezafibrate	[261] [258], [253], [339]
3-[(4-chlorobenzoyl) amino]propanoic acid	-
Bicalutamide	-
Candesartan	-
Carbamazepine	[261], [259], [253], [258], [260], [339]
2-Hydroxycarbamazepine	-
3-Hydroxycarbamazepine	-
10,11-Dihydro-10-hydroxycarbamazepine	-
10,11-Dihydroxy-10,11-dihydrocarbamazepine	-
Acridone	-
9-Acridine carboxylic acid	-
Cetirizine	[261]
Chlorothiazide	-
Citalopram	[258], [260]
Desmethylcitalopram	[260]
Didemethylcitalopram	-
Clarithromycin	[340]
Clopidogrel	[261], [260]
Clopidogrel carboxylic acid	[260]
Diclofenac	[253],[259], [261], [150], [339]
4'-Hydroxy-diclofenac	-
Diclofenac carboxylic acid	-
Diclofenac lactam	-
Diphenhydramine	[260]
N-Desmethyl diphenhydramine	-
Duloxetine	-
Fexofenadine	-
Flecainide	-
Flecainide-meta-O-dealkylated	-
Fluconazole	-
Fluoxetine	[256], [261], [258]
Norfluoxetine	[256]
Furosemide	[253]
Gabapentin	-
Gabapentin lactam	-
Hydrochlorothiazide	[253], [338]
Irbesartan	-
Lamotrigine	-
Levetiracetam	-
Levetiracetam acid	-
Lidocaine	-
Nor lidocaine	-
Metoprolol	[253], [258], [261]

Name	Reference
Hydroxy metoprolol	-
O-Desmethyl metoprolol	-
Naproxen	[261], [253]
O-Desmethyl naproxen	-
Olmesartan	-
Oxazepam	[256], [261], [260]
Phenytoin	-
Pregabalin	-
Primidone	[261]
Quetiapine	[260]
7-Hydroxy-quetiapine	-
Quetiapine sulfoxide	-
Ritalinic acid	-
Roxithromycin	-
Sertraline	[260], [339]
N-Desmethyl sertraline	[260]
Sertraline ketone	-
Sildenafil	[256], [261]
Sitagliptin	-
Sotalol	[253], [261]
Sulfamethoxazole	[259]
N-Acetyl sulfamethoxazole	-
Sulpiride	-
Tadalafil	-
Telmisartan	-
Torasemide	-
Hydroxytorasemide	-
Tramadol	[256]
Dehydrotramadol	-
O-Desmethyl tramadol	[256]
N,O-Didesmethyl tramadol	-
Trimethoprim	[253]
3-Desmethyl trimethoprim	-
5-(3,4,5-Trimethoxybenzoyl)-2,4-pyrimidinediamine	-
Valsartan	[338]
Valeryl-4-hydroxyvalsartan	-
Valsartanic acid	-
Venlafaxine	[256], [260]
N-Desmethyl venlafaxine	-
O-Desmethyl venlafaxine	-
N,O-Desmethyl venlafaxine	-
Xipamide	-
4-Acetamidoantipyrine	-
Abacavir	[261]
Acesulfame	[341]
Acyclovir	-
Bisoprolol	[258],[261]
Clindamycin	-
Clindamycin sulfoxide	-

Name	Reference
Emtricitabine	-
Emtricitabine carboxylate	-
Lamivudine	[261]
Metformin	-
Guanylurea	-

Table B.3: Sum formulas, suppliers and multiple reaction monitoring parameters of the analytes

Name	Formula	Supplier	Retention time [min]	MRM 1 (Quantification)	MRM 2 (Qualification)	DP [V]	CE [eV]	CXP [V]	Polarity
RPLC									
Aliskiren	$C_{30}H_{53}N_3O_6$	TRC	8.06	552.4/436.3	552.4/534.4	65	28/28	12/12	Positive
Amisulpride	$C_{17}H_{27}N_3O_4S$	TRC	5.51	370.2/242	370.2/196	106	39/59	14/12	Positive
Atenolol	$C_{14}H_{22}N_2O_3$	Sigma Aldrich	4.83	267/145	267/190	61	37/27	12/16	Positive
Atenolol acid	$C_{14}H_{21}NO_4$	TRC	5.14	268.1/191.2	268.1/226.1	56	27/25	16/20	Positive
Hydroxyatenolol	$C_{14}H_{22}N_2O_4$	TRC	4.68	283.1/116	283.1/74	65	25/40	8/8	Positive
Bezafibrate	$C_{19}H_{20}ClNO_4$	Sigma Aldrich	11.28	360.1/274.1	360.1/154	-65	-22/-36	-17/-9	Negative
3-[(4-chlorobenzoyl) amino]propanoic acid	$C_{10}H_{10}ClNO_3$	Sigma Aldrich	7.80	226/154.1	228/156.1	-45	-20/-20	-10/-10	Negative
Bicalutamide	$C_{18}H_{14}F_4N_2O_4S$	TRC	11.88	429.1/255	429.1/185	-55	-22/-50	-13/-9	Negative
Candesartan	$C_{24}H_{20}N_6O_3$	TRC	10.29	441.2/263.2	441.2/207.2	51	17/35	16/12	Positive
Carbamazepine	$C_{15}H_{12}N_2O$	Sigma Aldrich	9.37	237.1/194	237.1/179.1	71	27/49	16/12	Positive
2-Hydroxycarbamazepine	$C_{15}H_{12}N_2O_2$	Novartis	7.70	253.1/210.2	253.1/208	71	29/35	12/18	Positive
3-Hydroxycarbamazepine	$C_{15}H_{12}N_2O_2$	Novartis	8.13	253.1/210.1	253.1/167	66	27/51	14/10	Positive
10,11-Dihydro-10-hydroxycarbamazepine	$C_{15}H_{14}N_2O_2$	Novartis	7.40	255.2/194.1	255.2/179.1	46	27/52	14/14	Positive
10,11-Dihydroxy-10,11-dihydrocarbamazepine	$C_{15}H_{14}N_2O_3$	TRC	6.85	271/180	271/236	40	45/19	12/6	Positive
Acridone	$C_{13}H_9NO$	Th. Gever	8.79	196/167.1	196/139.1	96	43/71	30/22	Positive
9-Acridine carboxylic acid	$C_{14}H_9NO_2$	Santa Cruz	4.88	224.17/196.02	224.17/167	86	37/57	16/14	Positive
Cetirizine	$C_{21}H_{25}ClN_2O_3$	TRC	9.23	389.1/166.1	389.1/201.1	55	60/30	10/10	Positive
Chlorothiazide	$C_7H_6ClN_3O_4S_2$	Sigma Aldrich	5.60	294/179	294/214	-80	-62/-40	-10/-4	Negative
Citalopram	$C_{20}H_{21}FN_2O$	Labmix24	7.60	325.2/262.1	325.2/109.1	85	27/37	10/10	Positive
Desmethylcitalopram	$C_{19}H_{19}FN_2O$	TRC	7.49	311.1/262.1	311.1/109.1	45	26/32	10/10	Positive
Didemethylcitalopram	$C_{18}H_{17}FN_2O$	TRC	7.37	297.1/116	297.1/109	40	30/30	6/6	Positive
Clarithromycin	$C_{38}H_{69}NO_{13}$	Abbott	8.62	748.5/590.4	748.5/158.1	86	27/39	12/14	Positive
Clopidogrel	$C_{16}H_{16}ClNO_2S$	TRC	14.95	322.1/212	322.1/184	31	23/31	14/12	Positive
Clopidogrel carboxylic acid	$C_{15}H_{14}ClNO_2S$	TRC	6.98	308/198	308/152	66	23/33	12/10	Positive
Diclofenac	$C_{14}H_{10}Cl_2NO_2$	Sigma Aldrich	12.94	296/215	296/250	46	27/19	15/15	Positive
4'-Hydroxy-diclofenac	$C_{14}H_{11}Cl_2NO_3$	TRC	10.79	312/230	312/231	47	46/28	17/17	Positive

Name	Formula	Supplier	Retention time [min]	MRM 1 (Quantification)	MRM 2 (Qualification)	DP [V]	CE [eV]	CXP [V]	Polarity
Diclofenac carboxylic acid	$C_{13}H_9Cl_2NO_2$	TRC	13.19	282/229	282/264	28	37/14	16/14	Positive
Diclofenac lactam	$C_{14}H_9Cl_2NO$	TRC	12.70	278/214	278/215	60	39/30	16/13	Positive
Diphenhydramine	$C_{17}H_{21}NO$	TRC	7.49	256.2/167	256.2/152	20	20/50	5/8	Positive
N-Desmethyl diphenhydramine	$C_{16}H_{19}NO$	TRC	7.33	242/167	242/152	30	20/50	10/10	Positive
Duloxetine	$C_{18}H_{19}NOS$	EDQM	8.28	298.2/154	298.2/188	40	10/9	12/15	Positive
Fexofenadine	$C_{32}H_{39}NO_4$	TRC	8.52	502.3/466.3	502.3/171.1	80	38/57	5/5	Positive
Flecainide	$C_{17}H_{20}F_6N_2O_3$	TRC	7.82	415.2/398.1	415.2/301	80	35/50	10/10	Positive
Flecainide-meta-O-dealkylated	$C_{15}H_{19}F_3N_2O_3$	TRC	5.89	333.1/316.1	333.1/219.1	60	28/40	8/8	Positive
Fluconazole	$C_{13}H_{12}F_2N_6O$	TRC	6.78	307.1/238.1	307.1/220.1	70	20/25	20/15	Positive
Fluoxetine	$C_{17}H_{18}F_3NO$	Dr. Ehrenstorfer	8.70	310/44	310/148	45	30/14	10/10	Positive
Norfluoxetine	$C_{16}H_{16}F_3NO$	TRC	8.50	296/134	296/259	40	11/24	10/10	Positive
Furosemide	$C_{12}H_{11}ClN_2O_5S$	Sigma Aldrich	9.30	329/205	329/285	-90	-30/-20	-9/-13	Negative
Gabapentin	$C_9H_{17}NO_2$	TRC	5.07	172.1/137.2	172.1/154.2	55	22/19	10/10	Positive
Gabapentin lactam	$C_9H_{15}NO$	Sigma Aldrich	8.04	154.1/95	154.1/67	80	30/40	12/12	Positive
Hydrochlorothiazide	$C_7H_8ClN_3O_4S_2$	TRC	5.83	296/268.9	296/205	-120	-26/-32	-13/-11	Negative
Irbesartan	$C_{25}H_{28}N_6O$	TRC	11.02	427.2/193.1	427.2/121	-70	-35/-80	-6/-4	Negative
Lamotrigine	$C_9H_7Cl_2N_5$	TCI	5.94	256/211	256/157	80	38/45	10/10	Positive
Levetiracetam	$C_8H_{14}N_2O_2$	TRC	5.28	171.1/154.1	171.1/126.1	46	11/19	10/8	Positive
Levetiracetam acid	$C_8H_{13}NO_3$	TRC	5.77	172.1/126	172.1/69.2	96	19/33	8/8	Positive
Lidocaine	$C_{14}H_{22}N_2O$	TRC	5.71	235.2/86.1	235.2/58.1	80	23/53	14/2	Positive
Nor lidocaine	$C_{12}H_{18}N_2O$	TRC	5.38	207.1/58	207.1/122.1	35	30/20	8/8	Positive
Metoprolol	$C_{15}H_{25}NO_3$	Sigma Aldrich	6.05	268/116	268/74	75	27/35	10/11	Positive
Hydroxy metoprolol	$C_{15}H_{25}NO_4$	Sigma Aldrich	5.11	284.2/116	284.2/74	70	28/35	5/5	Positive
O-Desmethyl metoprolol	$C_{14}H_{23}NO_3$	TRC	5.11	254.2/116	254.2/177	70	25/25	8/10	Positive
Naproxen	$C_{14}H_{14}O_3$	Sigma Aldrich	11.18	229.1/170	229.1/185	-50	-22/-11	-11/-13	Negative
O-Desmethyl naproxen	$C_{13}H_{12}O_3$	Sigma Aldrich	8.71	215/169	215/171	-35	-40/-11	-8/-8	Negative
Olmesartan	$C_{24}H_{26}N_6O_3$	TRC	7.55	445.2/149.1	445.2/167.1	-50	-50/-35	-6/-6	Negative
Oxazepam	$C_{15}H_{11}ClN_2O_2$	Sigma Aldrich	9.78	287.1/241	287.1/104	61	47/81	8/6	Positive

Name	Formula	Supplier	Retention time [min]	MRM 1 (Quantification)	MRM 2 (Qualification)	DP [V]	CE [eV]	CXP [V]	Polarity
Phenytoin	$C_{15}H_{12}N_2O_2$	Sigma Aldrich	9.42	251/102	251/208	-45	-28/-25	-5/-5	Negative
Pregabalin	$C_8H_{17}NO_2$	TRC	5.09	160.1/97	160.1/55	41	21/35	5/10	Positive
Primidone	$C_{12}H_{14}N_2O_2$	Sigma Aldrich	6.63	219/162	219/91	40	16/39	13/13	Positive
Quetiapine	$C_{21}H_{25}N_3O_2S$	TCI	7.50	384.2/253.2	384.2/221.3	80	30/60	11/15	Positive
7-Hydroxy-quetiapine	$C_{21}H_{25}N_3O_3S$	TRC	5.82	400.1/269	400.1/208	80	35/65	5/14	Positive
Quetiapine sulfoxide	$C_{21}H_{25}N_3O_3S$	TRC	6.24	400/221	400/269	90	50/29	5/6	Positive
Ritalinic acid (Diastereoisomer 1)	$C_{13}H_{17}NO_2$	TRC	5.71	220.1/84.1	220.101/56	60	27/60	10/10	Positive
Ritalinic acid (Diastereoisomer 2)	$C_{13}H_{17}NO_3$	TRC	5.00	220.1/84.1	220.1/56.001	60	27/60	10/10	Positive
Roxithromycin	$C_{41}H_{76}N_2O_{15}$	Roussel-Uclaf	8.70	837.5/679.4	837.5/158	85	29/47	26/11	Positive
Sertraline	$C_{17}H_{17}Cl_2N$	Sigma Aldrich	8.74	306.3/275.1	306.3/159	63	20/37	7/13	Positive
Sertraline ketone	$C_{16}H_{12}Cl_2O$	TRC	15.57	291/145	291/117	80	29/40	10/8	Positive
Sitagliptin	$C_{16}H_{15}F_6N_5O$	TRC	6.40	408.1/235.1	408.1/174	51	29/33	38/24	Positive
Sotalol	$C_{12}H_{20}N_2O_3S$	Dr. Ehrenstorfer	4.89	273/134	273/213	46	37/26	10/10	Positive
Sulfamethoxazole	$C_{10}H_{11}N_3O_3S$	Sigma Aldrich	7.72	254.1/156	254.1/188	66	23/21	12/14	Positive
N-Acetyl sulfamethoxazole	$C_{12}H_{13}N_3O_4S$	EAWAG	7.92	296.1/198	296.1/134	81	25/35	14/12	Positive
Sulpiride	$C_{15}H_{23}N_3O_4S$	Sigma Aldrich	4.94	342.2/214	342.2/112.1	70	45/35	10/8	Positive
Tadalafil	$C_{22}H_{19}N_3O_4$	EDQM	10.31	388.1/262	388.1/232	-80	-26/-53	-6/-4	Negative
Telmisartan	$C_{33}H_{30}N_4O_2$	TRC	10.35	515.2/276.1	515.2/497.2	181	61/45	26/22	Positive
Torasemide	$C_{16}H_{20}N_4O_3S$	TRC	7.93	349.1/264.1	349.1/290.1	60	25/20	8/8	Positive
Hydroxytorasemide	$C_{16}H_{20}N_4O_4S$	TRC	5.92	365.1/280.1	365.1/306.1	50	25/20	8/8	Positive
Tramadol	$C_{16}H_{25}NO_2$	Fluka	6.05	264.2/58	n.a.	46	45	4/	Positive
Dehydrotramadol	$C_{16}H_{23}NO$	TRC	7.15	246.2/121	246.2/115	50	42/82	9/8	Positive
O-Desmethyl tramadol	$C_{15}H_{23}NO_2$	Sigma Aldrich	5.28	250.1/58	n.a.	45	20	11/	Positive
N,O-Didesmethyl tramadol	$C_{14}H_{21}NO_2$	TRC	5.33	236.1/44	n.a.	44	20	8/	Positive
Trimethoprim	$C_{14}H_{18}N_4O_3$	Sigma Aldrich	5.38	291.1/261.1	291.1/230.1	86	35/33	10/11	Positive
3-Desmethyl trimethoprim	$C_{13}H_{16}N_4O_3$	TRC	5.01	277.1/261.1	277.1/123.1	86	38/51	15/10	Positive
5-(3,4,5-Trimethoxybenzoyl)-2,4-pyrimidinediamine	$C_{14}H_{16}N_4O_4$	TRC	6.31	305.1/137	305.1/244.1	80	35/35	7/6	Positive

Name	Formula	Supplier	Retention time [min]	MRM 1 (Quantification)	MRM 2 (Qualification)	DP [V]	CE [eV]	CXP [V]	Polarity
Valsartan	$C_{24}H_{29}N_5O_3$	TRC	11.82	436.2/235.1	436.2/207.1	111	27/35	12/16	Positive
Valeryl-4-hydroxyvalsartan	$C_{24}H_{29}N_5O_4$	TRC	9.21	450.5/350	450.5/179	-75	-26/-40	-3/-8	Negative
Valsartanic acid	$C_{14}H_{10}N_4O_2$	TRC	8.18	267.1/206.1	267.1/151.1	80	17/57	10/10	Positive
Venlafaxine	$C_{17}H_{27}NO_2$	Promochem	6.77	278.2/58	278.2/121.1	36	43/28	8/8	Positive
N-Desmethyl venlafaxine	$C_{16}H_{25}NO_2$	Campro Scientific	6.65	264.1/44	264.1/121.1	36	55/37	11/10	Positive
O-Desmethyl venlafaxine	$C_{16}H_{25}NO_2$	Promochem	5.71	264.1/58	264.1/246	56	45/19	8/18	Positive
N,O-Desmethyl venlafaxine	$C_{15}H_{23}NO_2$	Campro Scientific	5.61	250.2/44.2	250.2/132.9	36	32/31	10/10	Positive
Xipamide	$C_{15}H_{15}ClN_2O_4S$	TRC	11.12	353.1/274.1	353.1/127	-60	-36/-45	-8/-8	Negative
HILIC									
4-Acetamidoantipyrine	$C_{13}H_{15}N_3O_2$	TRC	4.69	246.1/204	246.1/83	60	20/45	16/6	Positive
Abacavir	$C_{14}H_{18}N_6O$	Sigma Aldrich	5.43	287.2/191	287.2/79	31	25/47	30/12	Positive
Acyclovir	$C_8H_{11}N_5O_3$	TRC	10.81	226.1/152.1	226.1/135.1	71	17/43	12/14	Positive
Acesulfame	$C_4H_4NO_4S$	Sigma Aldrich	3.41	161.8/82	161.8/78	-50	-38/-22	-3/-5	Negative
Bisoprolol	$C_{18}H_{31}NO_4$	Merck	8.96	326.2/116	326.2/74	76	27/41	10/6	Positive
Clindamycin	$C_{18}H_{33}ClN_2O_5S$	TRC	12.50	425.2/126	425.2/377	70	50/28	6/11	Positive
Clindamycin sulfoxide	$C_{18}H_{34}Cl_2N_2O_6S$	TRC	14.61	441.2/377	441.2/126	55	26/41	11/6	Positive
Emtricitabine	$C_8H_{10}FN_3O_3S$	TRC	4.68	248.1/130	248.1/113	61	19/53	10/10	Positive
Lamivudine	$C_8H_{11}N_3O_3S$	LGC standard	6.95	230.1/112	230.1/95	56	19/51	18/6	Positive
Metformin	$C_4H_{11}N_5$	TRC	13.34	130.1/71	130.1/60	36	31/19	4/4	Positive
Guanylurea	$C_2H_6N_4O$	Sigma Aldrich	14.20	103.1/60	103.1/86	25	18/14	10/15	Positive

Table B.4: Suppliers and multiple reaction monitoring parameters of the surrogates

Name	Supplier	Retention time [min]	MRM	DP [V]	CE [eV]	CXP [V]	Polarity
RPLC							
Aliskiren-d_6	TRC	8.06	558.4/436.3	60	31	12	Positive
Amisulpride-d_5	TRC	5.51	375.2/242	106	39	10	Positive
Atenolol-d_7	Dr Ehrenstorfer	4.83	274/145	66	37	12	Positive
Hydroxyatenolol-d_7	TRC	4.68	290.1/123	60	38	8	Positive
Bezafibrate-d_4	TRC	11.28	364.1/158	-65	-38	-11	Negative
Bicalutamide-d_4	TRC	11.88	433.1/185	-55	-50	-8	Negative
Candesartan-d_5	TRC	10.29	446.2/268	66	17	14	Positive
Carbamazepine-$^{13}C,^{15}N$	Campro Scientific	9.37	239/192	61	29	12	Positive
10,11-Dihydro-10-hydroxycarbamazepine-d_3	TLC	7.40	258.2/240.1	26	15	16	Positive
Cetirizine-d_8	TRC	9.23	397.2/166.1	55	55	10	Positive
Chlorothiazide-$^{13}C,^{15}N_2$	TRC	5.60	297/216	-90	-40	-10	Negative
Citalopram-d_4	TRC	7.60	331.2/109.1	60	37	10	Positive
Didemethylcitalopram-d_6	TRC	7.37	303.2/266.1	60	22	6	Positive
Clarithromycin-N-methyl-d_3	TRC	8.62	751.5/161.2	70	40	8	Positive
Clopidogrel-d_4	TRC	14.95	326.1/216.1	31	23	10	Positive
Clopidogrel carboxylic acid-d_4	TRC	6.98	312/202	60	25	15	Positive
Diclofenac-d_4	Dr Ehrenstorfer	12.94	300/219	46	27	15	Positive
Diphenhydramine-d_6	TRC	7.49	262.2/152	30	55	8	Positive
N-Desmethyl diphenhydramine-d_3	TRC	7.33	245.2/167	30	20	8	Positive
Duloxetine-d_7	TRC	8.28	305/44	31	37	6	Positive
Fexofenadine-d_6	TRC	8.52	508.3/472.3	80	40	5	Positive
Flecainide-d_3	TRC	7.82	418.2/401.2	70	35	10	Positive
Fluconazole-d_4	TRC	6.78	311.1/223.1	70	25	17	Positive
Fluoxetine-d_5	Sigma-Aldrich	8.70	315/44	46	30	10	Positive
Norfluoxetine-d_5	Santa Cruz	8.50	301/139	31	11	10	Positive
Furosemide-d_5	TRC	9.30	334/206	-125	-34	-11	Negative

Name	Supplier	Retention time [min]	MRM	DP [V]	CE [eV]	CXP [V]	Polarity
Gabapentin-d_{10}	Sigma Aldrich	5.07	182.2/164.2	56	21	10	Positive
Gabapentin lactam-d_6	TRC	8.04	160.3/101.1	81	33	8	Positive
Hydrochlorothiazine-^{13}C,d_2	TRC	5.83	299/269.9	-130	-28	-13	Negative
Ibuprofen carboxylic acid-d_3	TRC	8.71	238/194	-28	-10	-6	Negative
Irbesartan-d_7	Santa Cruz Biotechnology	11.02	434.2/200.2	-70	-35	-6	Negative
Lamotrigine-^{13}C,$^{15}N_4$	Sigma Aldrich	5.94	261/46.1	86	79	4	Positive
Levetiracetam-d_6	TRC	5.28	177.1/132.1	36	20	11	Positive
Lidocaine-ethyl-d_{10}	TRC	5.71	245.2/96.1	96	23	12	Positive
Nor lidocaine-d_5	TRC	5.38	212.3/63.3	40	30	8	Positive
Metoprolol-d_7	Campro Scientific	6.05	275/123	80	27	16	Positive
Hydroxy metoprolol-d_5	TRC	5.11	289.2/121.1	80	28	8	Positive
O-Desmethyl metoprolol-d_5	TRC	5.11	259.2/182.2	70	28	10	Positive
Naproxen-d_3	Dr Ehrenstorfer	11.18	232/173	-30	-20	-5	Negative
Olmesartan-d_6	TRC	7.55	451.2/154.1	-45	-50	-6	Negative
Oxazepam-d_5	Sigma Aldrich	9.78	292.1/246	81	47	20	Positive
Pregabalin-d_4	CDN isotopes inc	5.09	164.1/85	64	21	6	Positive
Primidone-d_5	TRC	6.63	224/167.1	56	17	14	Positive
Quetiapine-d_8	TRC	7.50	392/258	70	35	20	Positive
Ritalinic acid-d_{10}	TRC	5.71	230.2/61.1	60	75	10	Positive
Roxithromycin-d_7	TRC	8.70	844.6/158	85	55	12	Positive
Sertraline-d_3	TRC	8.74	309/159	36	33	14	Positive
Sitagliptin-d_4	TRC	6.40	412.1/239.1	26	27	14	Positive
Sotalol-d_6	Dr Ehrenstorfer	4.89	279/214	46	25	10	Positive
Sulfamethoxazole-d_4	TRC	7.72	258/160	66	23	12	Positive
N-Acetyl sulfamethoxazole-d_4	TRC	7.92	300/202	81	23	18	Positive
Sulpiride-d_3	TRC	4.94	345.4/112.1	80	36	8	Positive
Tadalafil-d_3	TRC	10.31	391/262	-100	-27	-18	Negative
Telmisartan-d_3	TRC	10.35	518.3/500.2	171	47	20	Positive

Name	Supplier	Retention time [min]	MRM	DP [V]	CE [eV]	CXP [V]	Polarity
Torasemide-d_7	TRC	7.93	356.1/264.1	60	25	8	Positive
Hydroxytorasemide-d_7	TRC	5.92	372.1/306.1	55	20	8	Positive
O-Desmethyl tramadol-d_6	TRC	5.28	256.2/64	41	84	8	Positive
N,O-Didesmethyl tramadol-d_3	TRC	5.33	239.1/47	47	20	9	Positive
Trimethoprim-d_3	Sigma Aldrich	5.38	294/123.1	90	33	10	Positive
Valsartan-d_3	TRC	11.82	439.2/207.1	111	35	16	Positive
Valsartanic acid-d_4	TRC	8.18	271.1/210.1	80	17	10	Positive
O-Desmethyl venlafaxine-d_6	TRC	5.71	270.2/58	56	43	4	Positive
N,O-Desmethyl venlafaxine-d_3	TRC	5.61	253.2/47	48	47	8	Positive
Xipamide-d_6	TRC	11.12	359.1/78	-80	-55	-8	Negative
HILIC							
4-Acetamidoantipyrine-d_3	TRC	4.69	249.1/231.2	50	22	18	Positive
Abacavir-d_4	TRC	5.43	291.2/195	130	52	10	Positive
Acesulfame-d_4	TRC	3.41	165.74/86.1	-50	-22	-5	Negative
Acyclovir-d_4	TRC	10.81	230.1/152.1	46	19	12	Positive
Bisoprolol-d_7	TRC	8.96	333.3/123	90	26	6	Positive
Clindamycin-d_3	TRC	12.50	428.2/129	95	42	10	Positive
Emtricitabine-$^{13}C,^{15}N_2$	TRC	4.59	251/133	54	19	12	Positive
Lamivudine-$^{13}C,^{15}N_2$	TRC	6.95	233.1/115	95	20	9	Positive
Metformin-d_6	TRC	13.34	136/77.1	36	29	4	Positive
Guanylurea-$^{15}N_4$	TRC	14.20	107.1/63	40	17	10	Positive

Table B.5: Parameters of the LC-MS methods

	RPLC	HILIC
LC	Agilent 1260 (Waldbronn, Germany)	
Column guard	Zorbax Eclipse SB-C8 (2.1 × 12.5 mm, 5 µm, Agilent)	EC HILIC Nucleodur (4 × 3 mm, 3 µm, Machery-Nagel)
Column	Zorbax Eclipse Plus C18 column (2.1 ×150 mm, 3.5 µm, Agilent)	Zwitterionic HILIC Nucleodur (3 ×250 mm, 3 µm, Macherey-Nagel)
Injection volume	80 µL	70 µL
Flow rate	300 µL/min	500 µL/min
Eluent A	0.1% acetic acid	10 mM ammonium formiate, 0.1% formic acid
Eluent B	Acetonitrile	7.5 mM ammonium formiate in acetonitrile/Milli-Q, (90/10, v/v)
Gradient	0-1 min, 0% B 1-2 min 0-20% B 2-16 min, 20-100% B 16-19 min, 100% B 19-25 min, 0-100% B	0-3 min, 100% B 3-17 min 100-75% B 17-22 min, 75% B 22-33 min 100% B
Column temperature	25°C	
MS	Triple quadrupole (API 6500 QTrap, SCIEX, Darmstadt, Germany)	
Ion Source	Ion drive ™	
Ionization mode	Electrospray with polarity switch	
Curtain gas	45 psi	
Ion source gas 1	45 psi	
Ion source gas 2	40 psi	45 psi
Source temperature	450°C	500°C
Entrance potential	10 V (positive mode)/-10 V (negative mode)	
Ion spray voltage	5500 V (positive mode)/-4500 V (negative mode)	
MRM mode	Advanced scheduled MRM	
Target scan time	0.2 s (positive mode) 0.2 s (negative mode)	0.5 s (positive mode) 0.3 s (negative mode)
Post-divert valve to waste	0.0-2.0 min 17-25 min	0.0-2.0 min 22-33 min
MS data acquisition software	Analyst 1.6.2	

Table B.6: Analytes with their corresponding internal standards

Analytes	Internal standard
RPLC	
Aliskiren	Aliskiren-d_6
Amisulpride	Amisulpride-d_5
Atenolol	Atenolol-d_7
Atenolol acid	Atenolol-d_7
Hydroxyatenolol	Hydroxyatenolol-d_7
Bezafibrate	Bezafibrate-d_4
3-[(4-chlorobenzoyl) amino]propanoic acid	Bezafibrate-d_4
Bicalutamide	Bicalutamide-d_4
Candesartan	Candesartan-d_5
Carbamazepine	Carbamazepine-$^{13}C,^{15}N$
2-Hydroxycarbamazepine	Carbamazepine-$^{13}C,^{15}N$
3-Hydroxycarbamazepine	Carbamazepine-$^{13}C,^{15}N$
10,11-Dihydro-10-hydroxycarbamazepine	10,11-Dihydro-10-hydroxycarbamazepine-d_3
10,11-Dihydroxy-10,11-dihydrocarbamazepine	10,11-Dihydro-10-hydroxycarbamazepine-d_3
Acridone	Carbamazepine-$^{15}N,^{13}C$
9-Acridine carboxylic acid	Carbamazepine-$^{15}N,^{13}C$
Cetirizine	Cetirizine-d_8
Chlorothiazide	Chlorothiazide-$^{13}C,^{15}N_2$
Citalopram	Citalopram-d_4
Desmethylcitalopram	Citalopram-d_4
Didemethylcitalopram	Didemethylcitalopram-d_6
Clarithromycin	Clarithromycin-N-methyl-d_3
Clopidogrel	Clopidogrel-d_4
Clopidogrel carboxylic acid	Clopidogrel carboxylic acid-d_4
Diclofenac	Diclofenac-d_4
4'-Hydroxy-diclofenac	Diclofenac-d_4
Diclofenac carboxylic acid	Diclofenac-d_4
Diclofenac lactam	Diclofenac-d_4
Diphenhydramine	Diphenhydramine-d_6
N-Desmethyl diphenhydramine	N-Desmethyl diphenhydramine-d_3
Duloxetine	Duloxetine-d_7
Fexofenadine	Fexofenadine-d_6
Flecainide	Flecainide-d_3
Flecainide-meta-O-dealkylated	n.a
Fluconazole	Fluconazole-d_4
Fluoxetine	Fluoxetine-d_5
Norfluoxetine	Norfluoxetine-d_5
Furosemide	Furosemide-d_5
Gabapentin	Gabapentin-d_{10}
Gabapentin lactam	Gabapentin lactam-d_6
Hydrochlorothiazide	Hydrochlorothiazide-$^{13}C,d_2$
Irbesartan	Irbesartan-d_7
Lamotrigine	Lamotrigine-$^{13}C,^{15}N_4$
Levetiracetam	Levetiracetam-d_6
Levetiracetam acid	Levetiracetam-d_6
Lidocaine	Lidocaine-ethyl-d_{10}
Nor lidocaine	Nor lidocaine-d_5

Analytes	Internal standard
Metoprolol	Metoprolol-d_7
Hydroxy metoprolol	Hydroxy metoprolol-d_5
O-Desmethyl metoprolol	O-Desmethyl metoprolol-d_5
Naproxen	Naproxen-d_3
O-Desmethyl naproxen	Naproxen-d_3
Olmesartan	Olmesartan-d_6
Oxazepam	Oxazepam-d_5
Phenytoin	Ibuprofen carboxylic acid-d_3
Pregabalin	Pregabalin-d_4
Primidone	Primidone-d_5
Quetiapine	Quetiapine-d_8
7-Hydroxy-quetiapine	Quetiapine-d_8
Quetiapine sulfoxide	n.a.
Ritalinic acid (Diastereoisomer 1)	Ritalinic acid-d_{10}
Ritalinic acid (Diastereoisomer 2)	Ritalinic acid-d_{10}
Roxithromycin	Roxithromycin-d_7
Sertraline	Sertraline-d_3
Sertraline ketone	Diclofenac-d_4
Sitagliptin	Sitagliptin-d_4
Sotalol	Sotalol-d_6
Sulfamethoxazole	Sulfamethoxazole-d_4
N-Acetyl sulfamethoxazole	N-Acetyl sulfamethoxazole-d_4
Sulpiride	Sulpiride-d_3
Tadalafil	Tadalafil-d_3
Telmisartan	Telmisartan-d_3
Torasemide	Torasemide-d_7
Hydroxytorasemide	Hydroxytorasemide-d_7
Tramadol	n.a.
Dehydrotramadol	O-Desmethyl tramadol-d_6
O-Desmethyl tramadol	O-Desmethyl tramadol-d_6
N,O-Didesmethyl tramadol	N,O-Didesmethyl tramadol-d_3
Trimethoprim	Trimethoprim-d_3
3-Desmethyl trimethoprim	Trimethoprim-d_3
5-(3,4,5-Trimethoxybenzoyl)-2,4-pyrimidinediamine	Trimethoprim-d_3
Valsartan	Valsartan-d_3
Valeryl-4-hydroxyvalsartan	Valsartan-d_3
Valsartanic acid	Valsartanic acid-d_4
Venlafaxine	n.a.
N-Desmethyl venlafaxine	O-Desmethyl venlafaxine-d_6
O-Desmethyl venlafaxine	O-Desmethyl venlafaxine-d_6
N,O-Desmethyl venlafaxine	N,O-Desmethyl venlafaxine-d_3
Xipamide	Xipamide-d_6
HILIC	
4-Acetamidoantipyrine	4-Acetamidoantipyrine-d_3
Abacavir	Abacavir-d_4
Acyclovir	Acesulfame-d_4
Acesulfame	Acyclovir-d_4
Bisoprolol	Bisoprolol-d_7

Analytes	Internal standard
Clindamycin	Clindamycin-d_3
Clindamycin sulfoxide	n.a.
Emtricitabine	Emtricitabine-$^{13}C,^{15}N_2$
Lamivudine	Lamivudine-$^{13}C,^{15}N_2$
Metformin	Metformin-d_6
Guanylurea	Guanylurea-$^{15}N_4$

Table B.7: Characteristics of the Ehrenbreitstein sediment utilized for the sorption experiments

Properties	Measured values
pH (ratio L/S 1:5, in 0.01 M $CaCl_2$)	7.1
N [% dry weight]	0.37
TOC [% dry weight]	3.88
TC [% dry weight]	5.38
C/N	14.72

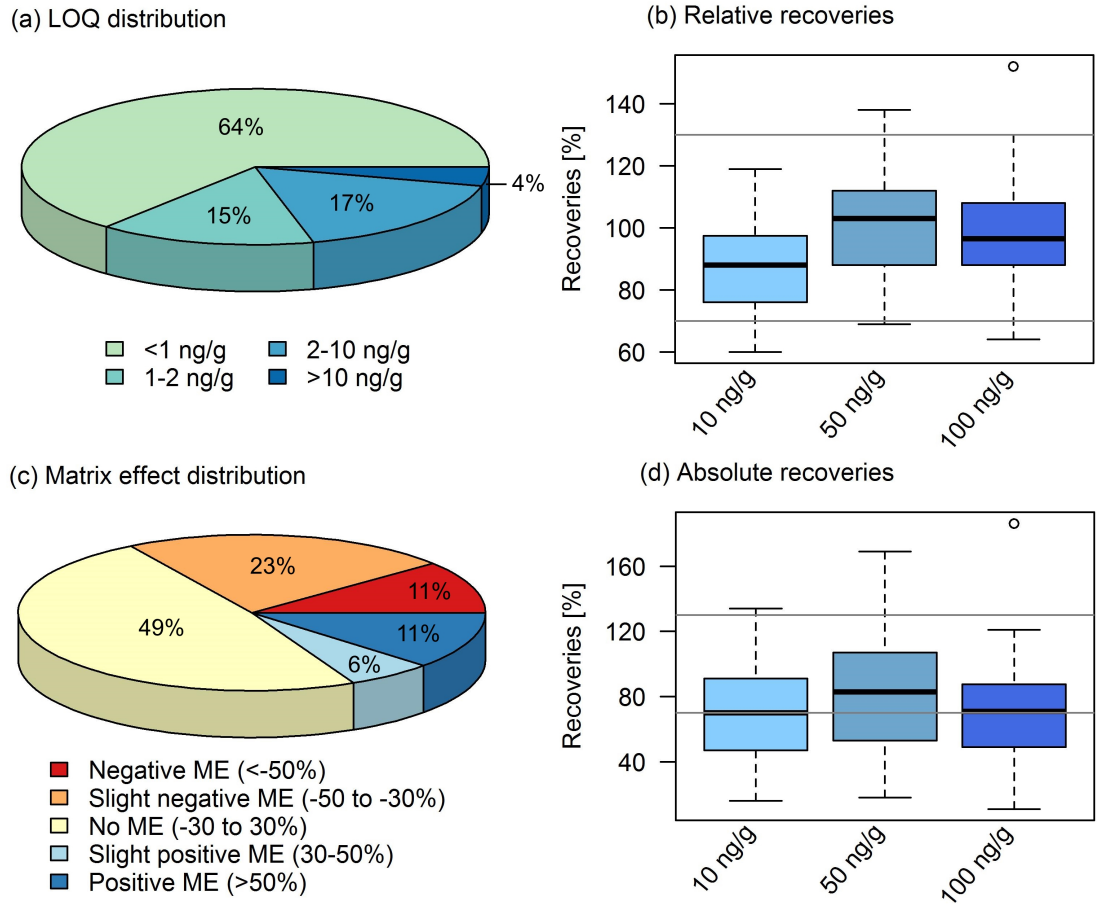


Figure B.1: Summary of the validation results. (a) Distribution of the limit of quantification. (b) Boxplot of the relative recoveries at the different tested spike levels. (c) Distribution of the matrix effects. (d) Boxplot of the absolute recoveries at the different tested spike levels. Gray lines correspond to recoveries of 70% and 130%, respectively.

Table B.8: Validation results

Analyte	Instrumental precison RSD [%]		Relative recoveries (n=4) [%]			Absolute recoveries (n=4) [%]			LOQ [ng/g]	Matrix effect [%]
	Intra-day (n=6)	Inter-day (n=4)	10 ng/g	50 ng/g	100 ng/g	10 ng/g	50 ng/g	100 ng/g		
RPLC										
Aliskiren	2.2	23.7	83 ± 8	73 ± 6	92 ± 11	n.a.	34 ± 14	114 ± 12	0.17	365 ± 37
Amisulpride	1.9	7.5	90 ± 21	105 ± 4	108 ± 15	124 ± 30	136 ± 6	112 ± 17	0.13	33 ± 6
Atenolol	3.1	3.7	100 ± 28	106 ± 8	99 ± 16	99 ± 31	107 ± 9	86 ± 13	0.83	-25 ± 4
Atenolol acid	7.8	6.1	97 ± 25	109 ± 7	81 ± 3	97 ± 30	111 ± 7	71 ± 6	2.23	-12 ± 8
Hydroxyatenolol	1.9	5.0	94 ± 16	110 ± 7	113 ± 16	21 ± 4	25 ± 1	24 ± 2	0.26	-70 ± 2
Bezafibrate	2.8	8.8	73 ± 13	86 ± 7	96 ± 15	54 ± 8	65 ± 3	53 ± 11	0.33	-38 ± 3
3-[(4-chlorobenzoyl) amino]propanoic acid	0.9	11.6	75 ± 11	84 ± 2	99 ± 8	55 ± 7	63 ± 2	55 ± 5	1.76	-43 ± 5
Bilcalutamide	1.0	0.9	101 ± 21	112 ± 13	106 ± 25	61 ± 11	67 ± 8	57 ± 15	0.28	-26 ± 5
Candesartan	0.8	7.5	82 ± 15	96 ± 8	95 ± 9	31 ± 4	37 ± 3	28 ± 5	1.67	-22 ± 5
Carbamazepine	1.9	3.1	87 ± 17	88 ± 13	94 ± 19	59 ± 11	65 ± 10	68 ± 17	0.34	-35 ± 4
2-Hydroxycarbamazepine	5.0	14.2	112 ± 27	112 ± 14	98 ± 17	76 ± 18	83 ± 10	70 ± 16	0.86	-18 ± 5
3-Hydroxycarbamazepine	1.8	7.4	76 ± 11	79 ± 10	91 ± 17	52 ± 7	58 ± 8	65 ± 16	0.81	-42 ± 2
10,11-Dihydro-10-hydroxycarbamazepine	5.6	5.5	75 ± 20	86 ± 11	82 ± 17	60 ± 15	69 ± 9	71 ± 16	1.58	-35 ± 3
10,11-Dihydroxy-10,11-dihydrocarbamazepine	4.4	7.3	86 ± 17	97 ± 10	82 ± 17	69 ± 14	78 ± 7	71 ± 17	0.44	-16 ± 5
Acridone	2.1	7.4	88 ± 21	88 ± 11	91 ± 14	57 ± 13	65 ± 8	65 ± 14	0.45	-43 ± 4
9-Acridine carboxylic acid	5.9	13.3	108 ± 20	116 ± 10	118 ± 12	71 ± 15	85 ± 7	81 ± 12	1.59	-26 ± 5
Cetirizine	1.5	8.6	115 ± 26	118 ± 9	130 ± 18	49 ± 4	54 ± 4	73 ± 6	1.50	-71 ± 2
Chlorothiazide	2.0	1.7	104 ± 17	113 ± 15	103 ± 9	72 ± 15	83 ± 9	68 ± 4	0.41	-33 ± 7
Citalopram	3.5	4.4	81 ± 21	99 ± 7	113 ± 13	51 ± 25	96 ± 12	116 ± 15	0.37	21 ± 6
Desmethylcitalopram	3.1	1.2	85 ± 19	98 ± 5	101 ± 9	78 ± 16	101 ± 6	107 ± 8	0.31	13 ± 8
Didemethylcitalopram	3.4	7.8	74 ± 19	84 ± 10	122 ± 14	46 ± 15	53 ± 6	55 ± 8	2.26	32 ± 10
Clarithromycin	3.7	14.4	65 ± 8	78 ± 5	73 ± 7	97 ± 23	118 ± 6	111 ± 15	0.41	287 ± 15
Clopidogrel	0.6	2.4	91 ± 19	96 ± 17	98 ± 8	45 ± 9	49 ± 9	60 ± 9	0.09	-21 ± 10
Clopidogrel carboxylic acid	0.6	2.3	91 ± 15	106 ± 9	102 ± 9	81 ± 16	96 ± 9	82 ± 6	0.33	-13 ± 5
Diclofenac	1.3	1.8	94 ± 20	106 ± 6	94 ± 16	43 ± 9	51 ± 3	45 ± 8	0.49	-55 ± 7

Analyte	Instrumental precison RSD [%]		Relative recoveries (n=4) [%]			Absolute recoveries (n=4) [%]			LOQ [ng/g]	Matrix effect [%]
	Intra-day (n=6)	Inter-day (n=4)	10 ng/g	50 ng/g	100 ng/g	10 ng/g	50 ng/g	100 ng/g		
4'-Hydroxy-diclofenac	1.0	5.8	93 ± 21	107 ± 7	81 ± 8	43 ± 10	52 ± 3	38 ± 4	0.72	-40 ± 5
Diclofenac carboxylic acid	1.1	21	99 ± 16	111 ± 4	95 ± 15	46 ± 8	53 ± 1	45 ± 8	0.35	-58 ± 8
Diclofenac lactam	1.3	0.4	67 ± 16	90 ± 14	78 ± 19	31 ± 7	43 ± 6	37 ± 9	0.24	-42 ± 12
Diphenhydramine	3.9	6.7	-[a]	107 ± 15	106 ± 15	32 ± 36	94 ± 14	109 ± 14	0.40	3 ± 21
N-Desmethyl diphenhydramine	3.8	6.5	119 ± 10	132 ± 10	89 ± 5	74 ± 10	86 ± 9	90 ± 5	0.97	-1 ± 7
Duloxetine	6.0	18.3	85 ± 11	82 ± 5	77 ± 10	96 ± 20	94 ± 2	77 ± 6	1.53	56 ± 11
Fexofenadine	2.9	8.2	119 ± 28	135 ± 11	102 ± 12	103 ± 22	117 ± 18	92 ± 12	0.72	-10 ± 6
Flecainide	2.2	17.5	76 ± 17	82 ± 3	113 ± 7	103 ± 26	113 ± 3	97 ± 10	0.14	25 ± 8
Flecainide-meta-O-dealkylated	2.7	26.7	-	-	-	107 ± 20	123 ± 5	102 ± 11	0.11	-9 ± 7
Fluconazole	3.8	3.1	92 ± 14	97 ± 13	99 ± 20	77 ± 12	89 ± 8	73 ± 19	0.33	-16 ± 6
Fluoxetine	3.1	9.4	74 ± 15	81 ± 6	88 ± 5	134 ± 40	154 ± 6	119 ± 11	0.21	304 ± 32
Norfluoxetine	5.9	1.5	72 ± 14	83 ± 8	86 ± 9	65 ± 13	78 ± 12	48 ± 5	0.51	347 ± 22
Furosemide	6.9	14.7	-[b]	105 ± 11	152 ± 18	-[b]	40 ± 4	42 ± 5	11.62	-49 ± 4
Gabapentin	4.4	5.3	68 ± 11	112 ± 10	85 ± 9	51 ± 12	73 ± 7	49 ± 9	5.66	-1 ± 7
Gabapentin lactam	2.9	6.5	79 ± 18	88 ± 12	94 ± 16	58 ± 12	65 ± 10	59 ± 15	1.42	-19 ± 5
Hydrochlorothiazide	6.5	6.3	85 ± 26	89 ± 6	99 ± 15	37 ± 8	41 ± 6	39 ± 1	0.45	-53 ± 5
Irbesartan	2.6	7.1	80 ± 18	88 ± 18	99 ± 8	16 ± 3	18 ± 3	11 ± 2	3.83	-38 ± 4
Lamotrigine	2.1	2.7	97 ± 17	117 ± 16	103 ± 17	56 ± 9	65 ± 6	49 ± 11	9.87	-33 ± 4
Levetiracetam	8.4	11.9	92 ± 9	97 ± 10	68 ± 16	75 ± 22	79 ± 13	68 ± 16	7.19	-32 ± 5
Levetiracetam acid	5.9	6.9	-[b]	91 ± 16	n.a.	-[b]	78 ± 7	n.a.	11.22	-17 ± 7
Lidocaine	1.3	3.5	105 ± 23	118 ± 13	113 ± 12	91 ± 18	104 ± 9	84 ± 6	0.23	-5 ± 5
Nor lidocaine	3.5	2.9	98 ± 20	109 ± 6	111 ± 11	73 ± 17	84 ± 4	73 ± 5	0.33	-17 ± 8
Metoprolol	3.0	2.5	92 ± 23	103 ± 7	86 ± 13	102 ± 25	117 ± 8	85 ± 10	0.77	-11 ± 6
Hydroxy metoprolol	4.5	8.0	99 ± 11	111 ± 12	109 ± 9	89 ± 16	100 ± 8	90 ± 9	0.69	-16 ± 4
O-Desmethyl metoprolol	4.5	5.7	117 ± 16	127 ± 11	113 ± 15	65 ± 12	76 ± 3	82 ± 9	1.08	-33 ± 6
Naproxen	7.1	9.4	90 ± 7	101 ± 9	108 ± 24	38 ± 6	47 ± 3	44 ± 9	4.41	-58 ± 4
O-Desmethyl naproxen	5.5	7.8	72 ± 7	78 ± 4	74 ± 13	31 ± 4	36 ± 1	30 ± 3	4.27	-59 ± 3
Olmesartan	2.6	2.4	82 ± 15	92 ± 11	93 ± 5	31 ± 5	37 ± 4	25 ± 3	2.34	-47 ± 5

Analyte	Instrumental precison RSD [%]		Relative recoveries (n=4) [%]			Absolute recoveries (n=4) [%]			LOQ [ng/g]	Matrix effect [%]
	Intra-day (n=6)	Inter-day (n=4)	10 ng/g	50 ng/g	100 ng/g	10 ng/g	50 ng/g	100 ng/g		
Oxazepam	2.7	2.7	102 ± 14	113 ± 4	91 ± 17	40 ± 7	46 ± 3	50 ± 8	4.35	-21 ± 5
Phenytoin	4.2	8.6	76 ± 19	81 ± 17	109 ± 23	37 ± 10	41 ± 6	38 ± 9	0.25	-55 ± 4
Pregabalin	11.5	5.3	71 ± 14	120 ± 15	82 ± 7	48 ± 5	85 ± 16	47 ± 6	8.27	4 ± 4
Primidone	3.9	8.4	76 ± 13	91 ± 15	96 ± 16	55 ± 7	66 ± 6	53 ± 11	1.11	-39 ± 4
Quetiapine	3.4	8.5	75 ± 12	85 ± 5	97 ± 7	71 ± 11	83 ± 11	86 ± 6	0.27	6 ± 7
7-Hydroxy quetiapine	5.3	16.2	95 ± 28	118 ± 22	n.a.	89 ± 22	112 ± 25	n.a.	0.83	65 ± 11
Quetipiane sulfoxide	2.1	4.5	-	-	-	110 ± 20	125 ± 2	110 ± 9	1.10	27 ± 8
Ritalinic acid (Diastereoisomer 1)	9.1	15.8	69 ± 7	83 ± 11	86 ± 9	99 ± 15	123 ± 15	81 ± 10	1.20	5 ± 3
Ritalinic acid (Diastereoisomer 2)	3.7	16.0	61 ± 12	72 ± 8	76 ± 9	87 ± 20	107 ± 10	73 ± 11	0.49	-2 ± 6
Roxithromycin	10.7	15.1	60 ± 4	78 ± 13	84 ± 42	133 ± 32	169 ± 9	186 ± 32	0.14	241 ± 48
Sertraline	3.6	25.9	67 ± 10	73 ± 7	90 ± 7	99 ± 22	120 ± 3	108 ± 12	1.44	84 ± 13
Sertraline ketone	3.4	40	116 ± 21	104 ± 16	87 ± 16	55 ± 9	51 ± 8	42 ± 8	8.86	-77 ± 3
Sitagliptin	1.1	1.0	97 ± 29	113 ± 10	89 ± 8	67 ± 37	95 ± 15	51 ± 6	0.24	-1 ± 6
Sotalol	3.2	3.5	91 ± 17	104 ± 9	89 ± 10	85 ± 19	101 ± 9	90 ± 11	0.78	-27 ± 5
Sulfamethoxazole	8.9	13.6	99 ± 26	95 ± 14	108 ± 30	19 ± 5	20 ± 2	22 ± 5	2.59	-40 ± 5
N-Acetyl sulfamethoxazole	9.4	10.2	96 ± 36	119 ± 11	108 ± 30	55 ± 9	67 ± 2	50 ± 10	3.58	-42 ± 4
Sulpiride	1.6	2.4	111 ± 23	122 ± 7	112 ± 16	104 ± 28	116 ± 2	107 ± 20	0.36	-16 ± 7
Tadalafil	1.4	2.2	76 ± 13	80 ± 13	94 ± 17	31 ± 5	33 ± 5	33 ± 5	0.34	-63 ± 3
Telmisartan	1.8	10.2	87 ± 27	94 ± 9	108 ± 9	112 ± 29	133 ± 16	121 ± 13	0.43	-3 ± 6
Torasemide	1.1	2.7	87 ± 17	103 ± 12	104 ± 12	82 ± 15	98 ± 11	72 ± 10	0.14	-7 ± 6
Hydroxytorasemide	2.8	6.6	88 ± 15	104 ± 6	99 ± 9	86 ± 16	102 ± 9	79 ± 10	0.17	1 ± 6
Tramadol	6.8	6.9	-	-	-	107 ± 11	118 ± 5	112 ± 16	0.37	2 ± 7
Dehydrotramadol	2.2	34.3	-[b]	138 ± 36	102 ± 4	99 ± 14	122 ± 33	93 ± 7	11.72	64 ± 8
O-Desmethyl tramadol	2.8	6.1	88 ± 24	103 ± 2	88 ± 14	74 ± 18	89 ± 5	80 ± 17	2.68	-34 ± 5
N,O-Didesmethyl tramadol	2.4	1.9	85 ± 15	103 ± 3	91 ± 20	79 ± 15	96 ± 6	79 ± 18	0.79	-26 ± 7
Trimethoprim	6.0	9.2	95 ± 20	119 ± 6	111 ± 17	68 ± 18	86 ± 8	77 ± 15	0.57	-26 ± 6
3-Desmethyl trimethoprim	3.5	9.5	104 ± 18	113 ± 4	116 ± 16	75 ± 14	82 ± 3	77 ± 11	0.20	-26 ± 4
5-(3,4,5-Trimethoxybenzoyl)-2,4-pyrimidinediamine	4.2	6.3	93 ± 18	103 ± 18	97 ± 19	70 ± 14	77 ± 12	67 ± 16	2.18	-27 ± 4

Analyte	Instrumental precison RSD [%]		Relative recoveries (n=4) [%]			Absolute recoveries (n=4) [%]			LOQ [ng/g]	Matrix effect [%]
	Intra-day (n=6)	Inter-day (n=4)	10 ng/g	50 ng/g	100 ng/g	10 ng/g	50 ng/g	100 ng/g		
Valsartan	3.7	5.9	99 ± 27	121 ± 18	111 ± 18	31 ± 8	39 ± 4	29 ± 5	4.56	-27 ± 6
Valeryl-4-hydroxyvalsartan	6.0	14.8	109 ± 16	111 ± 16	101 ± 15	34 ± 4	36 ± 2	27 ± 4	7.36	-37 ± 6
Valsartanic acid	12.8	16.8	87 ± 10	109 ± 43	91 ± 8	31 ± 2	39 ± 2	18 ± 2	1.25	-43 ± 4
Venlafaxine	1.6	5.6	-	-	-	88 ± 11	114 ± 8	116 ± 17	0.24	5 ± 7
N-Desmethyl venlafaxine	1.2	30.3	101 ± 15	122 ± 4	121 ± 12	112 ± 20	130 ± 4	111 ± 12	0.48	-15 ± 5
O-Desmethyl venlafaxine	1.6	1.0	93 ± 22	108 ± 6	94 ± 12	105 ± 29	115 ± 5	86 ± 15	0.24	-12 ± 6
N,O-Desmethyl venlafaxine	2.6	3.0	86 ± 14	99 ± 4	93 ± 12	81 ± 15	94 ± 3	81 ± 13	0.12	-22 ± 4
Xipamide	3.9	8.5	72 ± 13	83 ± 7	88 ± 11	83 ± 14	99 ± 8	84 ± 14	0.27	-15 ± 5
HILIC										
4-Acetamidoantipyrine	1.1	0.9	87 ± 27	93 ± 11	91 ± 16	47 ± 7	49 ± 6	49 ± 4	0.65	-43 ± 4
Abacavir	0.9	26.4	84 ± 21	87 ± 12	n.a.	46 ± 5	46 ± 6	n.a.	0.78	-50 ± 5
Acesulfame	4.2	15.1	70 ± 1	69 ± 1	71 ± 15	37 ± 6	44 ± 1	45 ± 1	0.44	-60 ± 3
Acyclovir	0.9	9.7	104 ± 37	114 ± 11	97 ± 7	97 ± 22	113 ± 15	90 ± 10	1.31	71 ± 18
Bisoprolol	0.8	2.4	82 ± 24	85 ± 11	110 ± 13	129 ± 19	128 ± 14	109 ± 13	0.26	33 ± 12
Clindamycin	1.4	1.7	84 ± 16	90 ± 11	88 ± 10	115 ± 10	122 ± 14	107 ± 16	0.60	69 ± 16
Clindamycin sulfoxide	2.0	13.6	-	-	-	88 ± 25	89 ± 8	86 ± 11	0.20	33 ± 13
Emtricitabine	5.6	5.0	66 ± 22	74 ± 6	64 ± 7	88 ± 15	96 ± 8	68 ± 4	1.40	16 ± 15
Lamivudine	1.5	3.4	90 ± 31	94 ± 10	92 ± 9	116 ± 19	117 ± 11	89 ± 11	0.90	34 ± 10
Metformin	1.7	1.3	-[a]	111 ± 19	101 ± 11	-[a]	158 ± 29	81 ± 5	4.90	37 ± 22
Guanylurea	0.9	17.4	-	-	-	-[a]	-[a]	69 ± 9	0.26	16 ± 15

[a] Environment concentration superior to spike level

[b] Spike level inferior to LOQ

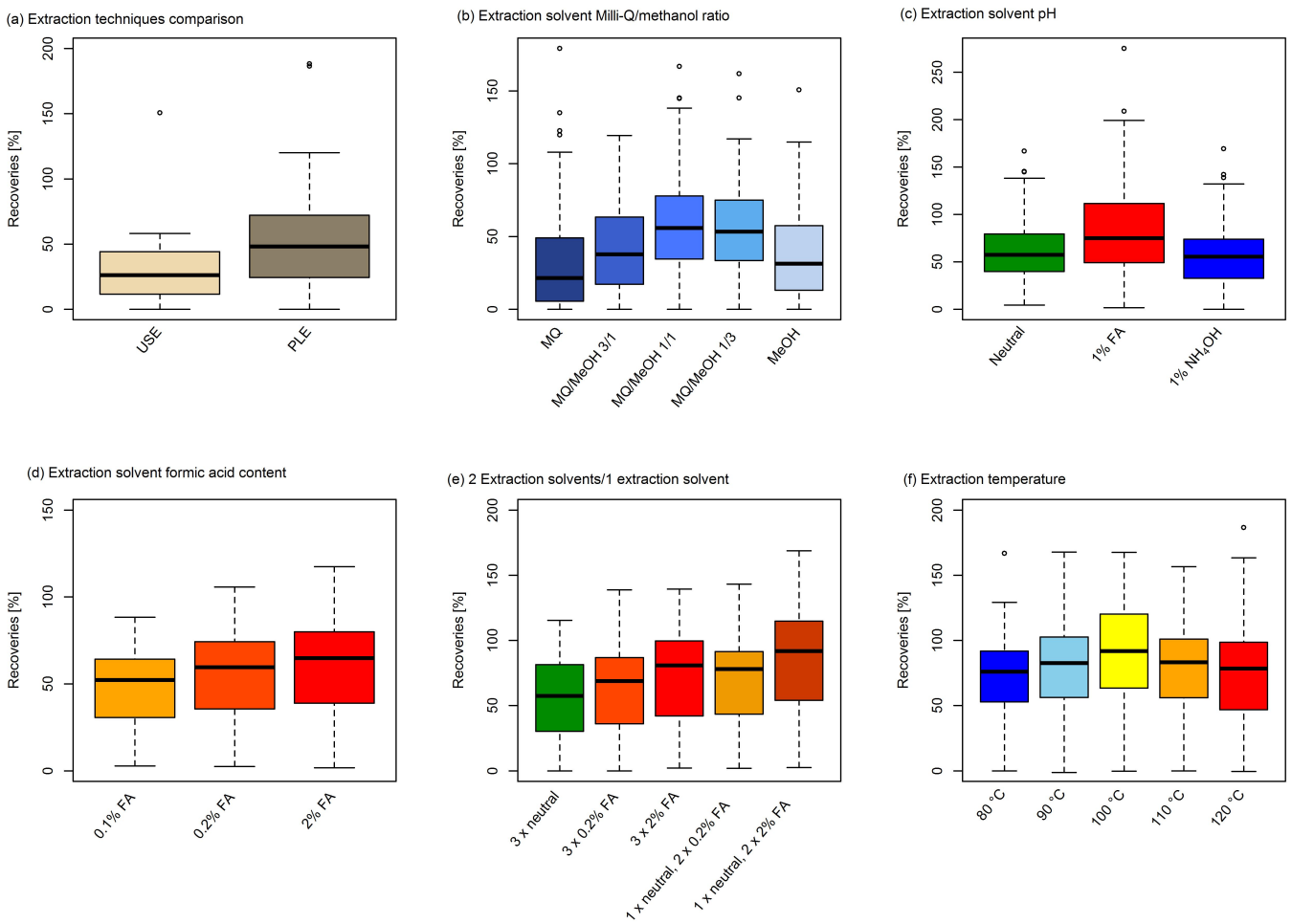


Figure B.2: Summary of the method development results. (a) Boxplot of the recoveries obtained with ultrason extraction (USE) and pressurized-liquid extraction (PLE). (b) Boxplot of the recoveries for different Milli-Q/methanol (MQ/MeOH) ratios of the extraction solvent. (c) Boxplot of the recoveries comparing neutral Milli-Q/methanol (1/1) as an extraction solvent with Milli-Q/methanol (1/1), 1% formic acid (FA) and Milli-Q/methanol (1/1) with 1% NH_4OH in the extraction solvent. (d) Boxplot of the recoveries comparing the addition of 0.1%, 0.2% and 1% formic acid in the extraction solvent Milli-Q/methanol (1/1). (e) Comparison of the recoveries with one and two extraction solvents and different addition in the solvent Milli-Q/methanol (1/1), 1x designed an extraction cycle. (f) Comparison of the extraction recoveries obtained at different extraction temperatures.

Table B.9: Sorption results

	Ratio solid to liquid 1:5					Ratio solid to liquid 1:25				
	K_d [L/kg]	R^2 (linear)	K_F [$\mu g^{1-n} L^n kg^{-1}$]	n	R^2 (Freundlich)	K_d [L/kg]	R^2 (linear)	K_F [$\mu g^{1-n} L^n kg^{-1}$]	n	R^2 (Freundlich)
RPLC										
Aliskiren	115 ± 2	0.999	150 ± 10	1.04 ± 0.02	0.999	149 ± 8	0.992	270 ± 40	1.07 ± 0.03	0.997
Amisulpride	119 ± 2	0.999	300 ± 40	1.18 ± 0.05	0.996	134 ± 3	0.999	240 ± 20	1.08 ± 0.02	0.999
Atenolol	24.6 ± 0.6[a]	0.999[a]	31 ± 7[a]	1.02 ± 0.04[a]	0.997[a]	19.9 ± 0.1	1.000	27 ± 2	1.03 ± 0.01	1.000
Atenolol acid	n.a	n.a	n.a	n.a	n.a	4.46 ± 0.08	0.999	10 ± 4	1.10 ± 0.06	0.991
Hydroxyatenolol	6.81 ± 0.03	1.000	10.1 ± 0.7	1.05 ± 0.01	1.000	8.9 ± 0.2	0.999	15 ± 1	1.06 ± 0.01	1.000
Bezafibrate	3.95 ± 0.07	0.999	5.2 ± 0.4	1.03 ± 0.01	1.000	4.57 ± 0.05	1.000	6.6 ± 0.5	1.04 ± 0.01	1.000
Bicalutamide	27.9 ± 0.5	0.999	35 ± 3	1.02 ± 0.01	0.999	31.7 ± 0.9	0.997	48 ± 5	1.04 ± 0.02	0.999
Candesartan	3.90 ± 0.06	0.999	4.7 ± 0.3	1.02 ± 0.01	1.000	7.6 ± 0.3	0.996	13 ± 5	1.06 ± 0.05	0.993
Carbamazepine	10.0 ± 0.2	0.999	15.3 ± 0.8	1.047 ± 0.009	1.000	11.7 ± 0.1	1.000	13 ± 1	1.01 ± 0.01	0.999
2-Hydroxycarbamazepine	11.0 ± 0.2	0.998	11 ± 4	0.98 ± 0.05	0.991	12.7 ± 0.2	0.999	15 ± 3	1.02 ± 0.03	0.998
3-Hydroxycarbamazepine	13.2 ± 0.3	0.998	13 ± 1	0.99 ± 0.02	0.999	14.74 ± 0.09	1.000	17 ± 3	1.02 ± 0.02	0.999
10-Hydroxy-10-hydroxycarbamazepine	2.53 ± 0.01	1.000	7 ± 1	1.12 ± 0.03	0.998	2.44 ± 0.06	0.998	3.8 ± 0.4	1.04 ± 0.01	0.999
10,11-Dihydroxy-10,11-dihydrocarbamazepine	2.91 ± 0.03	1.000	8 ± 2	1.14 ± 0.04	0.996	2.57 ± 0.05	0.999	6 ± 1	1.10 ± 0.03	0.998
Acridone	212 ± 5	0.998	180 ± 50	0.95 ± 0.06	0.989	191 ± 8	0.995	390 ± 40	1.09 ± 0.02	0.999
9-Acridine carboxylic acid	7.2 ± 0.2	0.998	11 ± 1	1.04 ± 0.01	0.999	3.75 ± 0.09	0.998	6.5 ± 0.7	1.05 ± 0.02	0.999
Chlorothiazide	7.53 ± 0.07	1.000	26 ± 9	1.18 ± 0.07	0.990	7.56 ± 0.04	1.000	12 ± 3	1.06 ± 0.03	0.997
Citalopram	2530 ± 40[a]	1.000[a]	1000 ± 300[a]	0.78 ± 0.08[a]	0.979[a]	5000 ± 200[a]	0.998[a]	3500 ± 800[a]	0.93 ± 0.06[a]	0.991[a]
Desmethylcitalopram	1070 ± 20[a]	0.999[a]	400 ± 200[a]	0.79 ± 0.09[a]	0.974[a]	1460 ± 60	0.994	1400 ± 200	0.97 ± 0.04	0.994
Didemethylcitalopram	n.a	n.a	n.a	n.a	n.a	900 ± 50[b]	0.997[b]	1200 ± 500[b]	1.04 ± 0.08[b]	0.994[b]
Clarithromycin	75 ± 0.6	1.000	70 ± 10	1.01 ± 0.03	0.997	177 ± 7	0.995	260 ± 60	1.05 ± 0.05	0.994
Clopidogrel	110 ± 1	1.000	120 ± 7	1.01 ± 0.01	1.000	112 ± 3	0.998	170 ± 10	1.05 ± 0.01	0.999
Clopidogrel carboxylic acid	3.84 ± 0.02	1.000	3.6 ± 0.3	0.99 ± 0.01	1.000	5.45 ± 0.08	0.999	5.1 ± 0.7	1.00 ± 0.02	0.999
Diclofenac	12.5 ± 0.1	1.000	14.1 ± 0.8	1.014 ± 0.009	1.000	n.a	n.a	n.a	n.a	n.a
4'-Hydroxy-diclofenac	15.5 ± 0.3	0.999	15 ± 1	0.99 ± 0.01	1.000	14.9 ± 0.2	1.000	15 ± 2	1.00 ± 0.01	0.999

	Ratio solid to liquid 1:5					Ratio solid to liquid 1:25				
	K_d [L/kg]	R^2 (linear)	K_F [$\mu g^{1-n} L^n kg^{-1}$]	n	R^2 (Freundlich)	K_d [L/kg]	R^2 (linear)	K_F [$\mu g^{1-n} L^n kg^{-1}$]	n	R^2 (Freundlich)
Diclofenac carboxylic acid	38.1 ± 0.1	1.000	40 ± 3	1.01 ± 0.01	0.999	33.3 ± 0.6	0.999	51 ± 6	1.05 ± 0.02	0.999
Diclofenac lactam	38.0 ± 0.8	0.999	38 ± 4	0.99 ± 0.02	0.999	40.9 ± 0.9	0.999	61 ± 5	1.04 ± 0.01	1.000
Diphenhydramine	n.a	n.a	n.a	n.a	n.a	9300 ± 700[a]	0.989[a]	3000 ± 1000[a]	0.8 ± 0.1[a]	0.968[a]
N-Desmethyl diphenhydramine	n.a	n.a	n.a	n.a	n.a	1190 ± 70	0.990	n.a.	n.a.	n.a.
Fexofenadine	72.9 ± 0.5	1.000	89 ± 5	1.03 ± 0.01	1.000	87 ± 1	0.999	120 ± 10	1.04 ± 0.02	0.999
Flecainide	77.3 ± 0.7	1.000	92 ± 3	1.02 ± 0.006	1.000	102 ± 3	0.998	190 ± 30	1.08 ± 0.03	0.998
Flecainide-meta-O-dealkylated	29.2 ± 0.7	0.998	39 ± 3	1.03 ± 0.01	0.999	30.7 ± 0.9	0.997	57 ± 6	1.07 ± 0.02	0.999
Fluconazole	5.5 ± 0.1	0.998	10.0 ± 0.7	1.06 ± 0.01	1.000	8.0 ± 0.4	0.994	25 ± 6	1.13 ± 0.04	0.996
Fluoxetine	n.a	n.a	n.a	n.a	n.a	4100 ± 300[a]	0.990[a]	60000 ± 20000[a]	3.0 ± 0.9[a]	0.849[a]
Norfluoxetine	n.a	n.a	n.a	n.a	n.a	3000 ± 200	0.991	1900 ± 500	0.88 ± 0.06	0.985
Furosemide	8.86 ± 0.05	1.000	8 ± 1	0.99 ± 0.02	0.998	9.2 ± 0.2	0.998	8 ± 1	0.98 ± 0.02	0.999
Gabapentin	1.6 ± 0.008	1.000	3.9 ± 0.8	1.09 ± 0.03	0.998	1.90 ± 0.01	1.000	6 ± 1	1.11 ± 0.02	0.999
Gabapentin lactam	2.196 ± 0.006	1.000	10 ± 3	1.18 ± 0.05	0.995	2.57 ± 0.01	1.000	11 ± 3	1.17 ± 0.05	0.996
Hydrochlorothiazide	4.26 ± 0.04	1.000	6 ± 1	1.04 ± 0.02	0.998	5.1 ± 0.1	0.999	7 ± 1	1.03 ± 0.02	0.999
Irbesartan	15.3 ± 0.2	0.999	17 ± 3	1.00 ± 0.02	0.998	57 ± 4	0.984	180 ± 60	1.15 ± 0.07	0.990
Lamotrigine	36.6 ± 0.9	0.998	44 ± 9	1.02 ± 0.04	0.996	31 ± 1	0.996	34 ± 9	1.00 ± 0.04	0.996
Levetiracetam	0.86 ± 0.04	0.992	n.a.	n.a.	n.a.	1.09 ± 0.09[a]	0.986[a]	n.a.	n.a.	n.a.
Levetiracetam acid	0.69 ± 0.05[b]	0.994[b]	n.a.	n.a.	n.a.	0.74 ± 0.02	0.998	0.10 ± 0.06	0.82 ± 0.05	0.988
Lidocaine	5.72 ± 0.08	0.999	8.6 ± 0.2	1.044 ± 0.004	1.000	6.9 ± 0.2	0.997	11 ± 2	1.04 ± 0.02	0.999
Nor lidocaine	4.78 ± 0.02	1.000	9 ± 1	1.08 ± 0.03	0.998	6.14 ± 0.07	1.000	11 ± 1	1.07 ± 0.02	0.999
Hydroxy metoprolol	7.4 ± 0.1	0.999	37 ± 7	1.22 ± 0.04	0.997	10.6 ± 0.3	0.998	30 ± 7	1.13 ± 0.04	0.997
Naproxen	5.75 ± 0.03	1.000	9 ± 1	1.06 ± 0.03	0.998	6.29 ± 0.06	1.000	10 ± 3	1.06 ± 0.04	0.996
Olmesartan	2.117 ± 0.009	1.000	1.9 ± 0.1	0.984 ± 0.008	1.000	4.3 ± 0.3	0.985	12 ± 4	1.11 ± 0.05	0.993
Oxazepam	30.9 ± 0.6	0.999	110 ± 20	1.19 ± 0.05	0.995	38.5 ± 1	0.998	84 ± 10	1.09 ± 0.02	0.999
Phenytoin	11.0 ± 0.2	0.999	13 ± 2	1.01 ± 0.03	0.998	10.7 ± 0.2	0.999	18 ± 1	1.058 ± 0.009	1.000
Pregabalin	1.42 ± 0.04	0.997	1.2 ± 0.8	0.99 ± 0.08	0.980	1.85 ± 0.03	0.999	9 ± 4	1.21 ± 0.07	0.989
Primidone	1.35 ± 0.01	1.000	2.1 ± 0.1	1.046 ± 0.009	1.000	1.73 ± 0.01	1.000	2.7 ± 0.2	1.05 ± 0.01	1.000

	Ratio solid to liquid 1:5					Ratio solid to liquid 1:25				
	K_d [L/kg]	R^2 (linear)	K_F [$\mu g^{1-n} L^n kg^{-1}$]	n	R^2 (Freundlich)	K_d [L/kg]	R^2 (linear)	K_F [$\mu g^{1-n} L^n kg^{-1}$]	n	R^2 (Freundlich)
Quetiapine	n.a	n.a	n.a	n.a	n.a	290 ± 20[b]	0.997[b]	800 ± 200[b]	1.14 ± 0.04[b]	0.999[b]
Quetiapine sulfoxide	133 ± 2	0.999	120 ± 30	0.96 ± 0.05	0.991	91 ± 2	0.998	150 ± 20	1.06 ± 0.03	0.998
Ritalinic acid (diastereoisomere 1)	1.90 ± 0.04	0.999	4 ± 2	1.08 ± 0.06	0.990	2.4 ± 0.03	0.999	9 ± 4	1.17 ± 0.08	0.987
Ritalinic acid (diastereoisomere 2)	1.38 ± 0.04[b]	0.999[b]	3.4 ± 0.4[b]	1.1 ± 0.02[b]	1.000[b]	1.69 ± 0.04[a]	0.999[a]	1.8 ± 0.4[a]	1.01 ± 0.03[a]	0.999[a]
Roxithromycin	77 ± 2	0.999	60 ± 20	0.94 ± 0.07	0.983	192 ± 9	0.994	340 ± 40	1.07 ± 0.03	0.998
Sertraline	n.a	n.a	n.a	n.a	n.a	2710 ± 50[b]	1.000[b]	3800 ± 100[b]	1.066 ± 0.009[b]	1.000[b]
Sitagliptin	441 ± 4	1.000	700 ± 70	1.11 ± 0.04	0.997	540 ± 20	0.995	1100 ± 200	1.12 ± 0.05	0.995
Sotalol	6.53 ± 0.08	1.000	3 ± 2	0.90 ± 0.07	0.984	8.7 ± 0.08	1.000	n.a.	n.a.	n.a.
Sulfamethoxazole	3.09 ± 0.04[b]	1.000[b]	11 ± 2[b]	1.15 ± 0.03[b]	0.999[b]	3.3 ± 0.1[a]	0.998[a]	200 ± 100[a]	1.8 ± 0.3[a]	0.960[a]
N-Acetyl sulfamethoxazole	1.5 ± 0.2[b]	0.989[b]	n.a.	n.a.	n.a.	1.58 ± 0.05[b]	0.999[b]	2.5 ± 0.7[b]	1.05 ± 0.03[b]	0.999[b]
Sulpiride	35.5 ± 0.8	0.999	29 ± 9	0.96 ± 0.05	0.991	42 ± 1	0.997	110 ± 10	1.12 ± 0.02	0.999
Tadalafil	81 ± 1[a]	1.000[a]	131 ± 7[a]	1.07 ± 0.01[a]	1.000[a]	83 ± 2	0.998	160 ± 20	1.08 ± 0.02	0.999
Telmisartan	850 ± 50[a]	0.993[a]	7000 ± 3000[a]	2.2 ± 0.6[a]	0.868[a]	580 ± 30	0.994	1500 ± 200	1.16 ± 0.04	0.997
Torasemide	5.19 ± 0.02	1.000	5.4 ± 0.2	1.002 ± 0.006	1.000	5.7 ± 0.2	0.997	9 ± 1	1.04 ± 0.02	0.998
Tramadol	19.7 ± 0.4	0.999	29 ± 5	1.04 ± 0.03	0.998	23.7 ± 0.4	0.999	19 ± 5	0.97 ± 0.03	0.996
Dehydrotramadol	81 ± 1[a]	0.999[a]	46 ± 8[a]	0.92 ± 0.03[a]	0.998[a]	116 ± 7	0.991	50 ± 30	0.88 ± 0.08	0.978
O-Desmethyl tramadol	8.55 ± 0.07	1.000	3.4 ± 0.3	0.908 ± 0.009	1.000	11.2 ± 0.5	0.994	10 ± 20	1.1 ± 0.2	0.909
N,O-Didesmethyl tramadol	6.3 ± 0.1	0.999	16 ± 3	1.12 ± 0.03	0.998	8.8 ± 0.2	0.999	9 ± 1	0.99 ± 0.02	0.999
Trimethoprim	167 ± 6[a]	0.998[a]	270 ± 30[a]	1.07 ± 0.03[a]	0.998[a]	161 ± 6	0.996	480 ± 30	1.15 ± 0.02	0.999
3-Desmethyl trimethoprim	147 ± 3	0.999	180 ± 20	1.02 ± 0.03	0.998	131 ± 5	0.996	280 ± 30	1.10 ± 0.02	0.999
5-(3,4,5-Trimethoxybenzoyl)-2,4-pyrimidinediamine	21.6 ± 0.6	0.998	31 ± 5	1.03 ± 0.03	0.998	20.9 ± 0.8	0.995	53 ± 6	1.10 ± 0.02	0.999
Valsartan	2.70 ± 0.02	1.000	10 ± 4	1.18 ± 0.08	0.986	5.6 ± 0.1	0.998	17 ± 6	1.14 ± 0.06	0.992
Valsartanic acid	1.90 ± 0.04	0.999	3.5 ± 0.4	1.07 ± 0.02	0.999	2.96 ± 0.07	0.999	5 ± 1	1.06 ± 0.04	0.996
Venlafaxine	163 ± 2	1.000	169 ± 6	1.004 ± 0.008	1.000	212 ± 5	0.999	310 ± 40	1.05 ± 0.02	0.998
N-Desmethyl venlafaxine	30.59 ± 0.08	1.000	36 ± 2	1.02 ± 0.01	1.000	47.8 ± 0.4	1.000	70 ± 8	1.05 ± 0.02	0.999

	Ratio solid to liquid 1:5					Ratio solid to liquid 1:25				
	K_d [L/kg]	R^2 (linear)	K_F [$\mu g^{1-n} L^n kg^{-1}$]	n	R^2 (Freundlich)	K_d [L/kg]	R^2 (linear)	K_F [$\mu g^{1-n} L^n kg^{-1}$]	n	R^2 (Freundlich)
O-Desmethyl venlafaxine	45.6 ± 0.2	1.000	15 ± 6	0.87 ± 0.05	0.991	72.8 ± 0.8	1.000	70 ± 10	1.00 ± 0.03	0.997
N,O-Desmethyl venlafaxine	12.0 ± 0.2	0.999	15 ± 1	1.03 ± 0.01	1.000	14.1 ± 0.2	0.999	18 ± 3	1.02 ± 0.03	0.998
Xipamide	9.8 ± 0.2	0.999	12.3 ± 0.9	1.02 ± 0.01	1.000	8.8 ± 0.2	0.999	12.2 ± 0.8	1.032 ± 0.009	1.000
HILIC										
4-Acetylaminoantipyrine	1.08 ± 0.003	1.000	1.46 ± 0.06	1.033 ± 0.005	1.000	1.494 ± 0.006	1.000	1.2 ± 0.2	0.97 ± 0.02	0.999
Abacavir	9.5 ± 0.1	0.999	12 ± 2	1.02 ± 0.02	0.998	8.9 ± 0.2	0.998	15 ± 3	1.06 ± 0.03	0.998
Acesulfame	0.640 ± 0.006	1.000	0.7 ± 0.3	1.00 ± 0.05	0.994	0.986 ± 0.002	1.000	2.2 ± 0.7	1.08 ± 0.04	0.996
Aciclovir	4.85 ± 0.03	1.000	22 ± 5	1.21 ± 0.05	0.996	5.6 ± 0.2	0.997	16 ± 1	1.11 ± 0.01	0.999
Bisoprolol	14.7 ± 0.2	1.000	18 ± 1	1.02 ± 0.01	1.000	21.6 ± 0.5	0.998	34 ± 4	1.05 ± 0.02	0.999
Clindamycin	13.28 ± 0.09	1.000	15 ± 1	1.01 ± 0.01	1.000	20.5 ± 0.5	0.998	30 ± 4	1.04 ± 0.02	0.999
Clindamycin sulfoxide	10.97 ± 0.08	1.000	10.2 ± 0.4	0.994 ± 0.006	1.000	14.3 ± 0.3	0.998	36 ± 9	1.12 ± 0.04	0.995
Emtricitabine	2.8 ± 0.1	0.995	3 ± 1	0.97 ± 0.06	0.988	2.31 ± 0.02	1.000	1.8 ± 0.4	0.97 ± 0.03	0.997
Lamivudine	2.41 ± 0.01	1.000	1.9 ± 0.6	0.97 ± 0.04	0.995	3.11 ± 0.03[a]	1.000[a]	4.4 ± 0.8[a]	1.04 ± 0.02[a]	0.999[a]
Metformin	8.2 ± 0.3	0.996	n.a.	n.a.	n.a.	8.5 ± 0.2	0.999	17 ± 10	1.08 ± 0.09	0.978
Guanylurea	14.0 ± 0.1	1.000	11 ± 4	0.97 ± 0.04	0.996	17.2 ± 0.6	0.996	47 ± 5	1.09 ± 0.01	1.000

[a] One point was taken off because under LOQ

[b] Two points were taken off because under LOQ

Table B.10: Comparison of the determined Kd values with those reported in the literature

Name	Kd [L/kg]	Type of solid phase	TOC [%]	Reference
Atenolol	1.3 ± 0.3	River sediment	0.075	[252]
	8.1 ± 0.6	River sediment	0.87	[252]
	5.3 ± 1.0	River sediment	1.7	[252]
	110 ± 0	Soil	2.2	[252]
	1.13	River sediment	0.74	[250]
	3.1	River sediment	4.36	[250]
	7.93	Groundwater sediment	1.44	[251]
	24.6 ± 0.6	River sediment	3.88	Our Study
	19.9 ± 0.1	River sediment	3.88	Our Study
Carbamazepine	0.085 ± 0.014	River sediment	0.075	[252]
	1.4 ± 0	River sediment	0.87	[252]
	1.8 ± 0.3	River sediment	1.7	[252]
	12 ± 1	Soil	2.2	[252]
	1.7	River sediment	0.74	[249]
	12.3	River sediment	4.36	[249]
	0.4	Groundwater sediment	1.44	[251]
	10.0 ± 0.2	River sediment	3.88	Our Study
	11.7 ± 0.1	River sediment	3.88	Our Study
10,11-dihydroxy-10,11-dihydrocarbamazepine	0.2	River sediment	0.74	[249]
	1.8	River sediment	4.36	[249]
Diclofenac	1.21 ± 0.36	Agricultural soil	0.44	[342]
	3.47 ± 0.73	Soil	0.55	[342]
	17.72 ± 7.45	Soil	1.43	[342]
	2.83 ± 1.05	Soil	3.16	[342]
	12.5 ± 0.1	River sediment	3.88	Our Study
Fluoxetine	18 ± 0.3	River sediment	0.075	[252]
	490 ± 40	River sediment	0.87	[252]
	4300 ± 400	River sediment	1.7	[252]
	2400 ± 100	Soil	2.2	[252]
	4100 ± 300	River sediment	3.88	Our Study
Naproxen	1.86	Groundwater sediment	1.44	[251]
	1.24 ± 0.31	Soil	0.44	[342]
	1.65 ± 0.52	Soil	0.55	[342]
	16.49 ± 5.17	Soil	1.43	[342]
	6.99 ± 2.33	Soil	3.16	[342]
	5.75 ± 0.03	River sediment	3.88	Our Study
	6.29 ± 0.06	River sediment	3.88	Our Study
Oxazepam	2.0	River sediment	0.74	[249]
	23.5	River sediment	4.36	[249]
	30.9 ± 0.6	River sediment	3.88	Our Study
	38.5 ± 1	River sediment	3.88	Our Study
Sotalol	1.41	River sediment	0.74	[250]
	3.9	River sediment	4.36	[250]
	6.53 ± 0.08	River sediment	3.88	Our Study
	8.7 ± 0.08	River sediment	3.88	Our Study
Sulfamethoxazole	0.2	River sediment	0.74	[249]
	0.9	River sediment	4.36	[249]

Name	Kd [L/kg]	Type of solid phase	TOC [%]	Reference
	4.25	Groundwater sediment	1.44	[251]
	3.09 ± 0.04	River sediment	3.88	Our Study
	3.3 ± 0.1	River sediment	3.88	Our Study
N-Acetyl sulfamethoxazole	0.013	River sediment	0.74	[249]
	0.7	River sediment	4.36	[249]
	1.5 ± 0.2	River sediment	3.88	Our Study
	1.58 ± 0.05	River sediment	3.88	Our Study
Tramadol	2.4	River sediment	0.74	[249]
	7.7	River sediment	4.36	[249]
	19.7 ± 0.4	River sediment	3.88	Our Study
	23.7 ± 0.4	River sediment	3.88	Our Study
Bisoprolol	2.00	River sediment	0.74	[250]
	6.5	River sediment	4.36	[250]
	14.7 ± 0.2	River sediment	3.88	Our Study
	21.6 ± 0.5	River sediment	3.88	Our Study
Metformin	3-2079	Soil	0.88-4.80	[343]
	8.2 ± 0.3	River sediment	3.88	Our Study
	8.5 ± 0.2	River sediment	3.88	Our Study

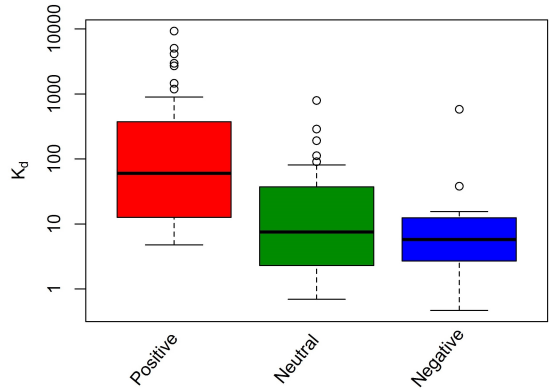


Figure B.3: Boxplot of the experimentally determined K_d for the positively charged, neutral and negatively charged analytes (at pH 7)

Table B.11: TOC-values at the different sampling locations for each annual composite sample

	TOC [%][a]										
Sampling location	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
Saar. Staustufe Rehlingen (Saartal)	n.a.	8.2	6.6	6.7	7.1	6.3	5.8	6.8	5.2	6.0	6.1
Weil (km 174) (Oberrhein)	3.7	3.1	3.6	4.1	4.5	3.9	4.2	4.4	2.5	2.9	2.5
Iffezheim (km 334) (Raum Seltz/Iffezheim)	3.5	3.9	3.9	3.1	4.3	4.2	4.3	4.1	3.8	3.4	2.8
Koblenz (km 590.3) (Oberhalb Moselmündung)	2.4	3.4	3.2	3.5	3.9	3.9	3.8	4.0	2.9	2.6	2.6
Bimmen (km 865) (Niederrhein)	4.0	4.7	4.4	3.7	4.2	4.3	4.2	4.5	4.0	3.9	3.7

[a] Data provided by the online information platform of the German Environmental Specimen Bank (https://www.umweltprobenbank.de/)

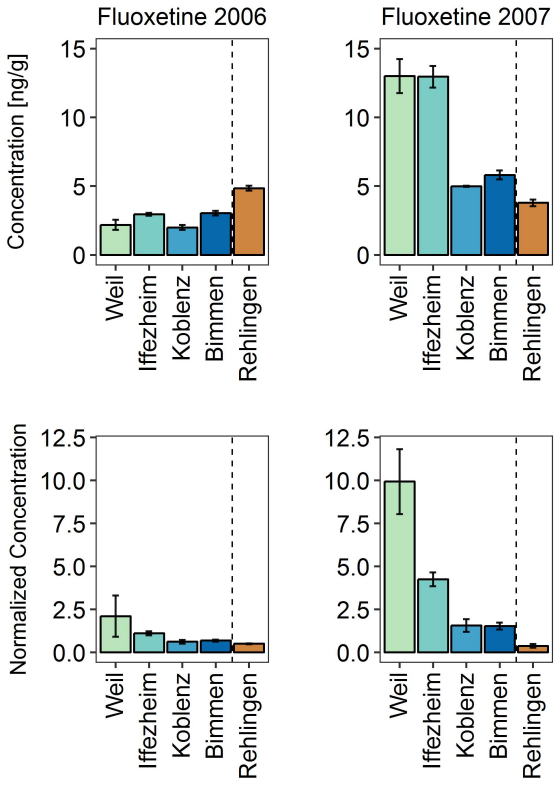


Figure B.4: Concentrations and normalized concentrations (against carbamazepine) for fluoxetine at the different sample sites for the years 2006 and 2007. Error bars correspond to the 95% confidence interval.

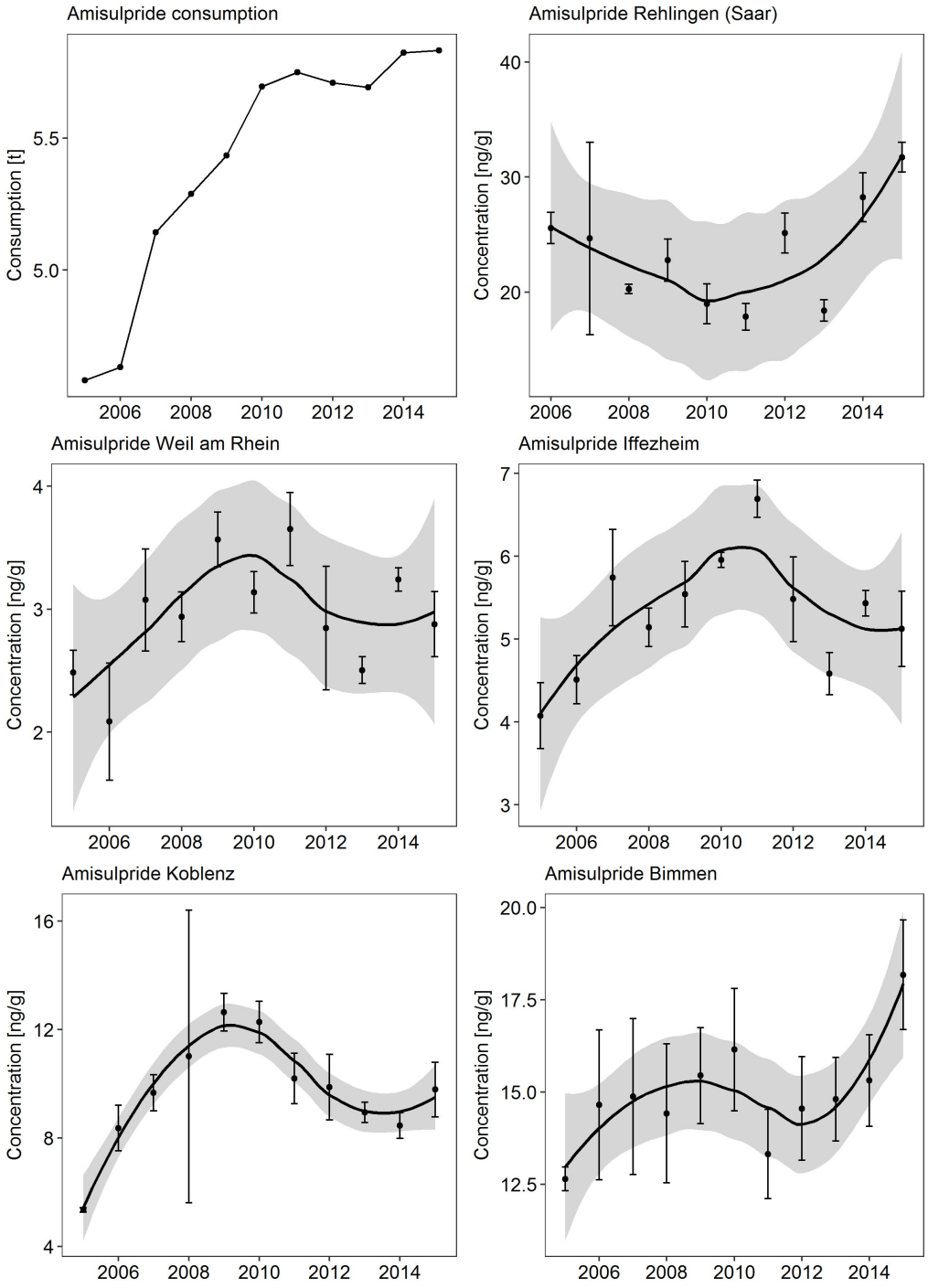
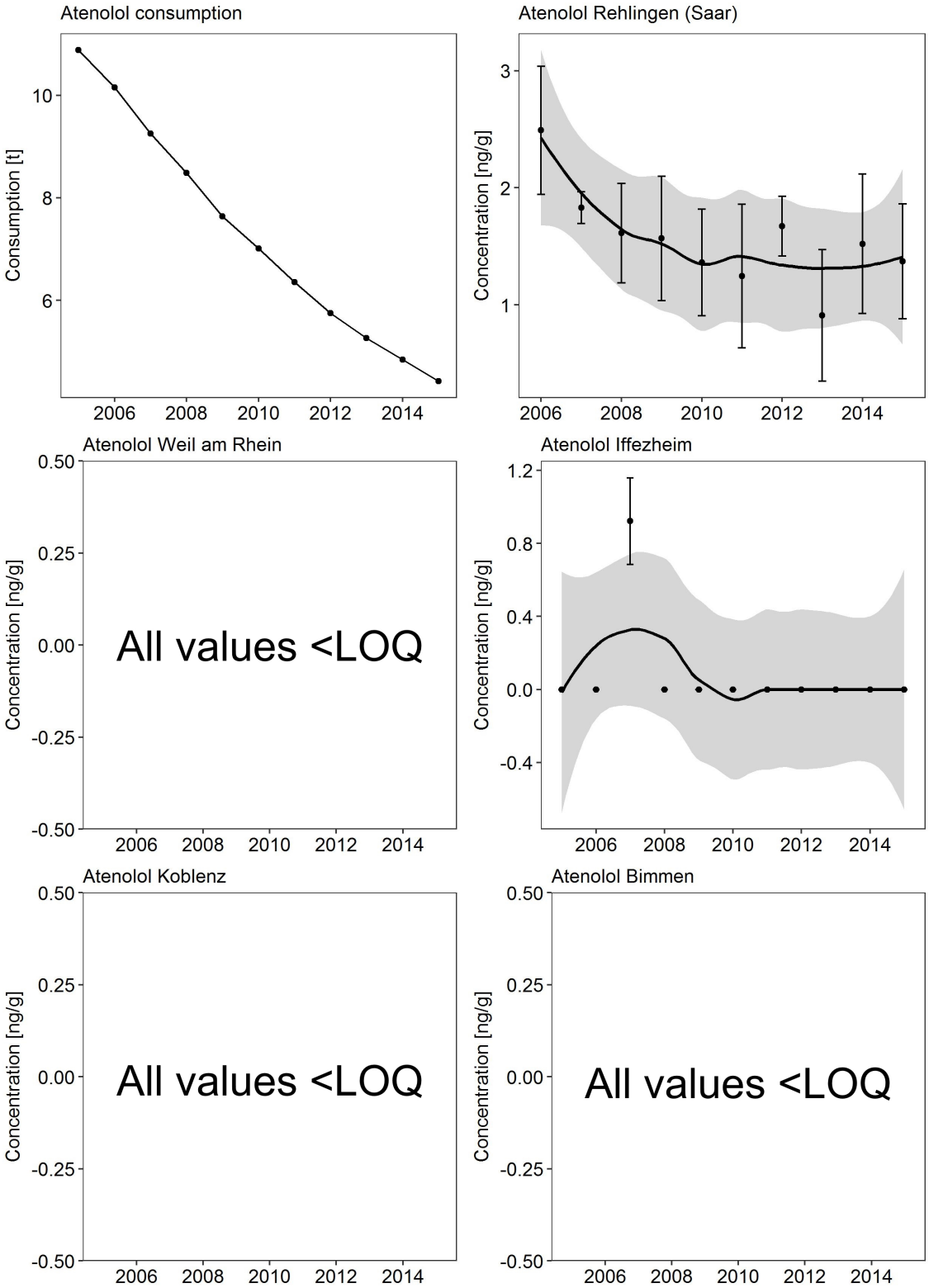


Figure B.5: Consumption and temporal concentration trends for the different analytes in SPM from the sampling sites Weil am Rhein (Rhine, km 173), Iffezheim (Rhine, km 333), Koblenz (Rhine, km 590), Bimmen (Rhine, km 863), Rehlingen (Saar, km 54). The grey shaded area corresponds to the pointwise 95% confidence interval of the LOESS function. Measurements were performed in triplicate and the error bars correspond to the 95% confidence interval. Consumption data: IQVIA Ltd. (2017) Midas© database (Frankfurt am Main, Germany).



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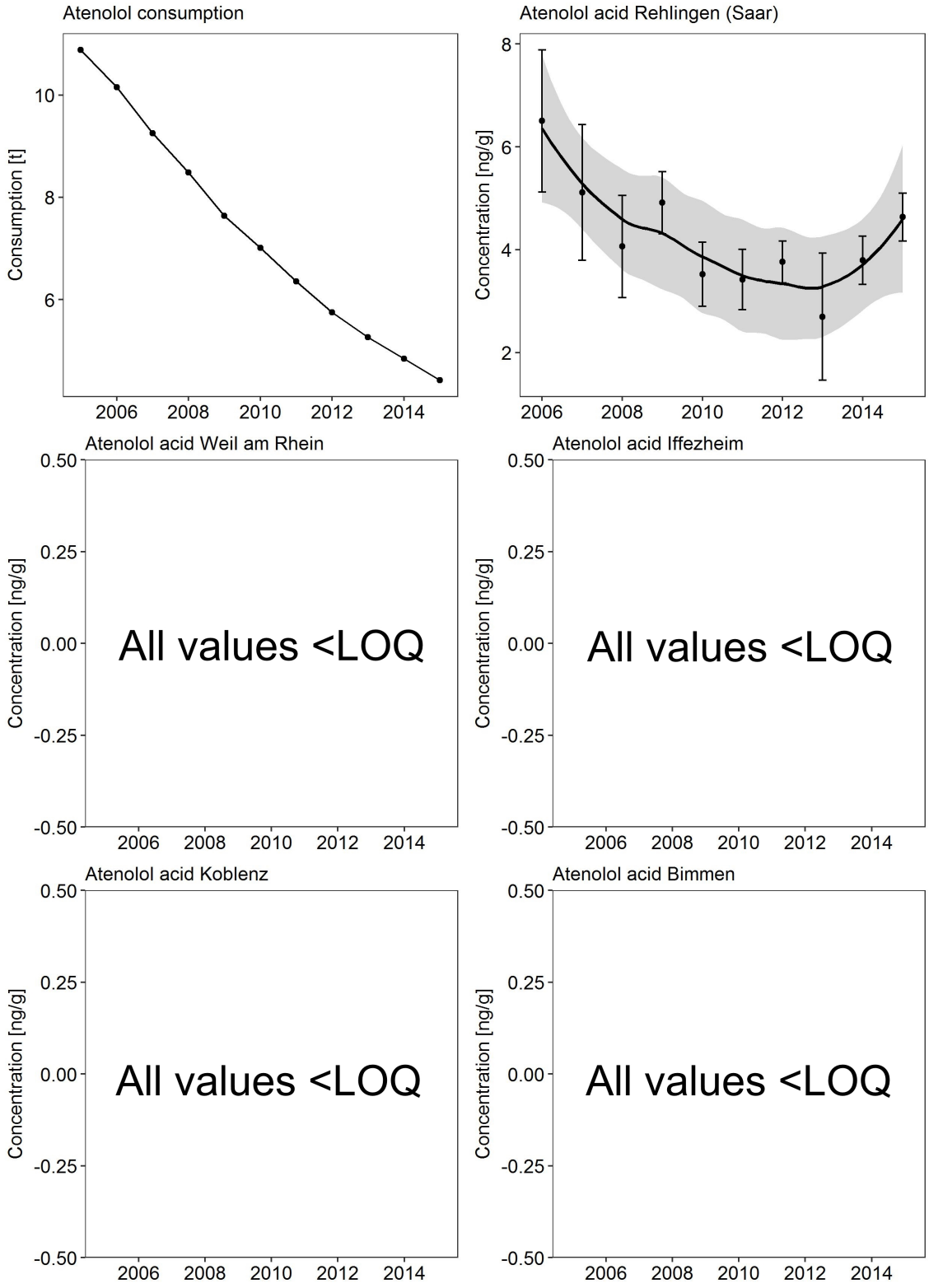

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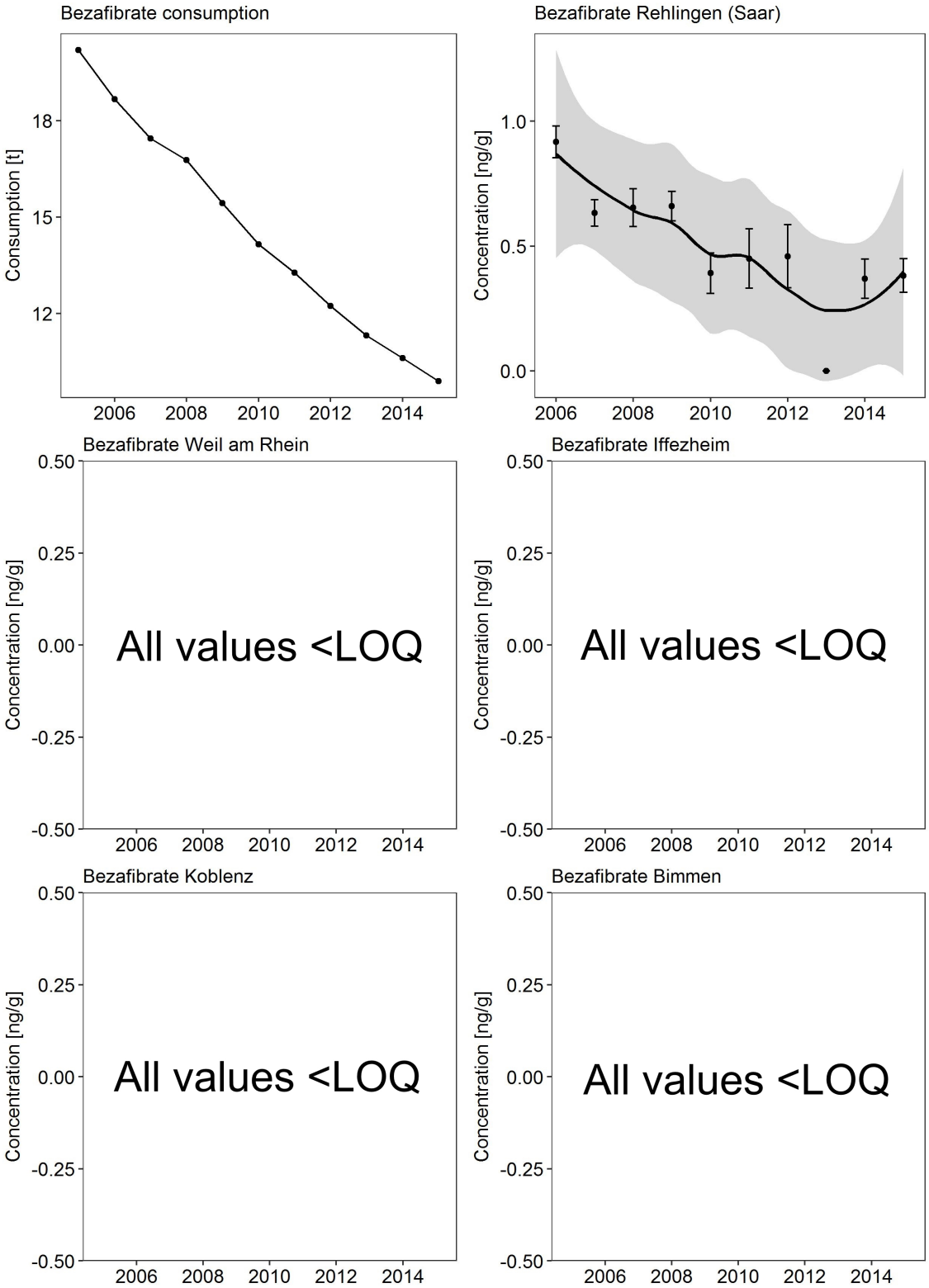

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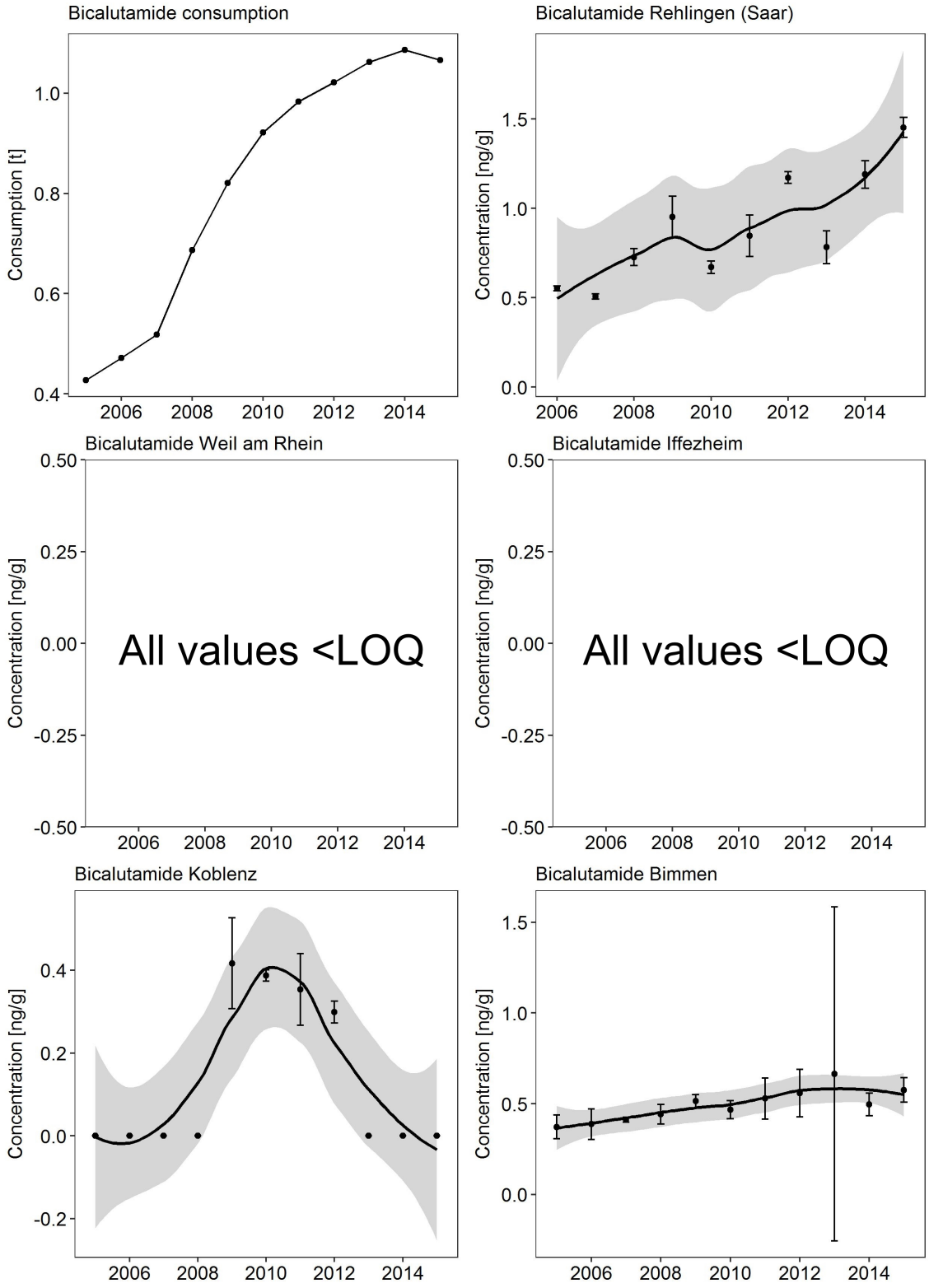

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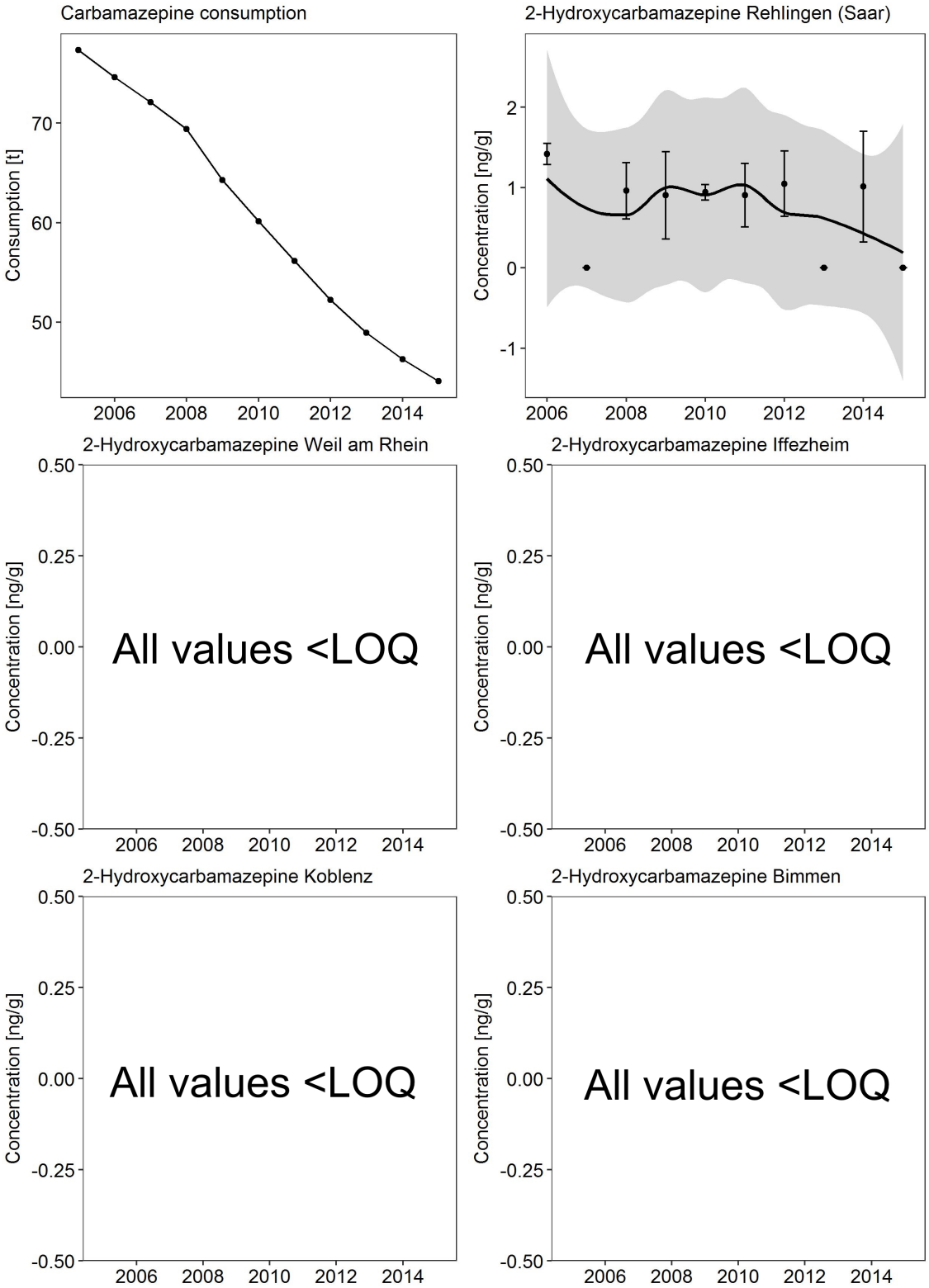

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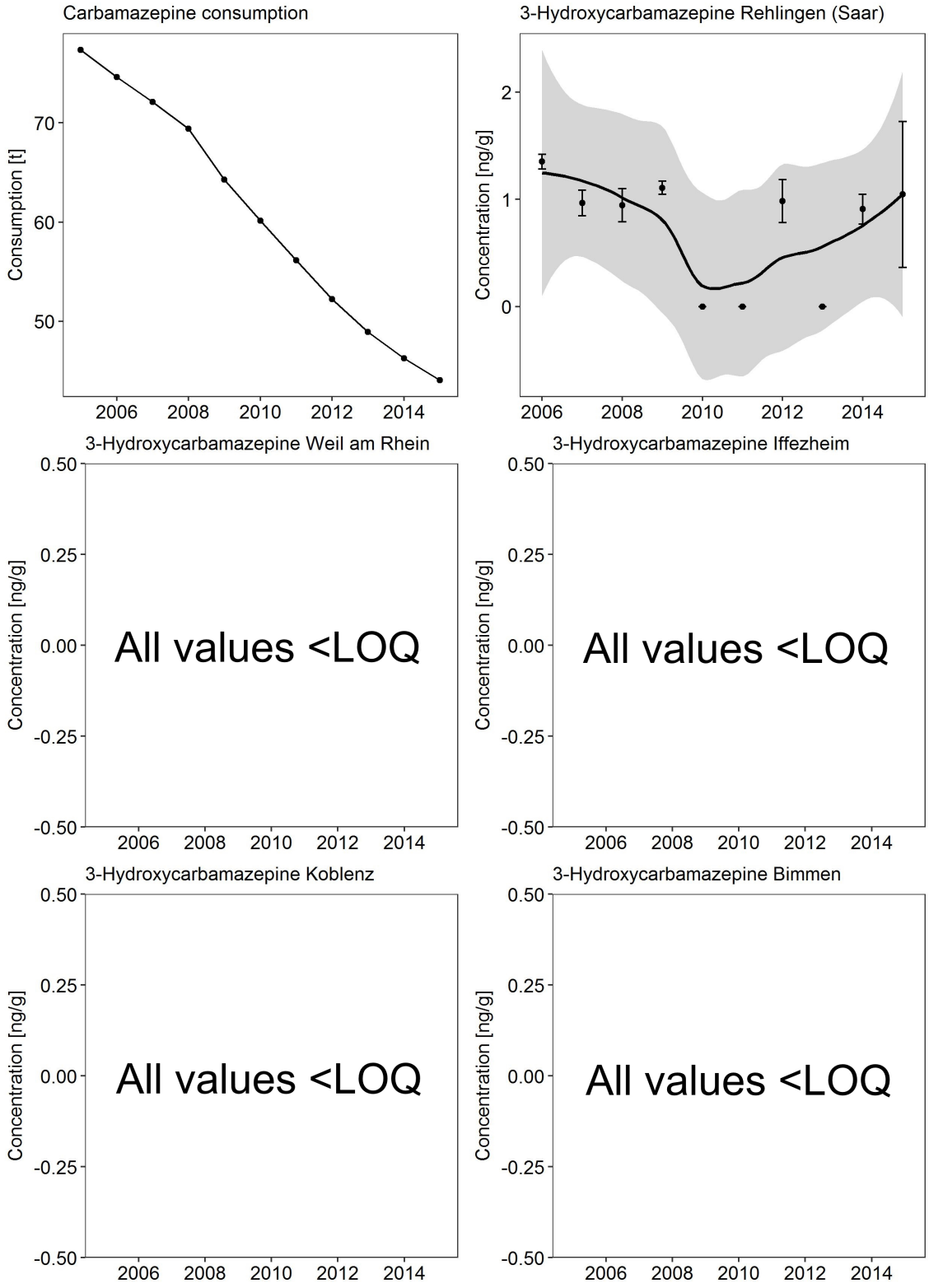

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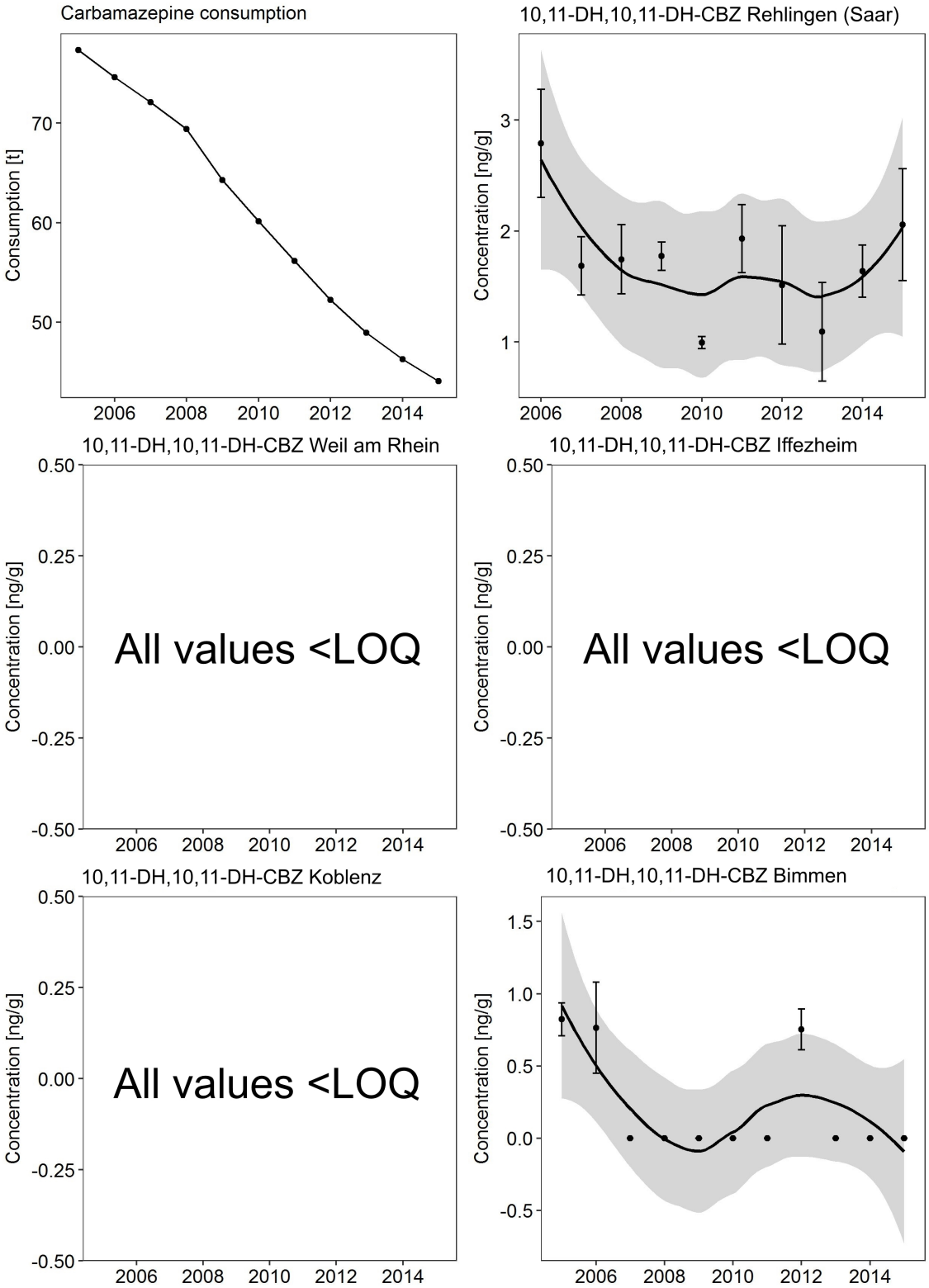

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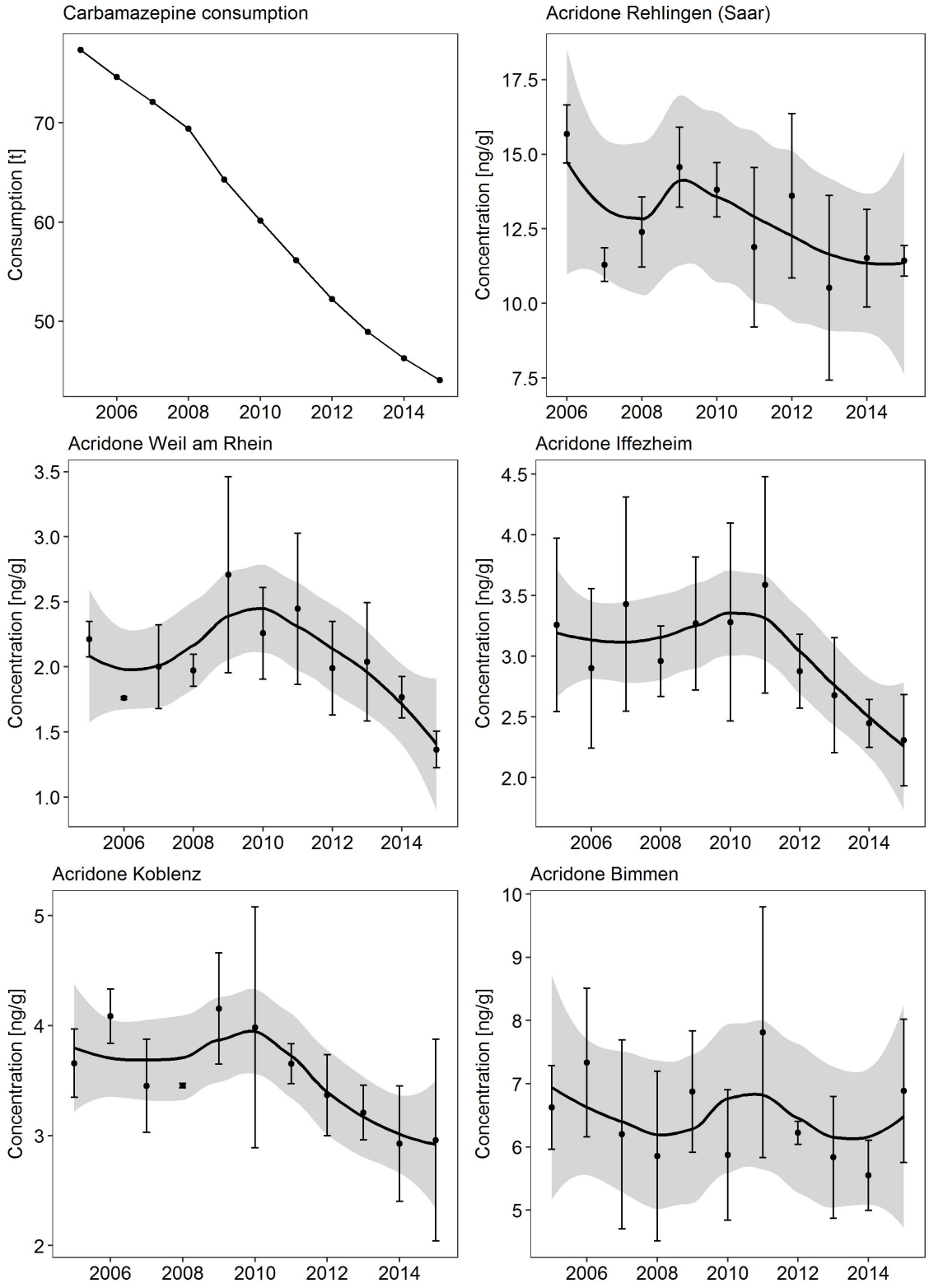

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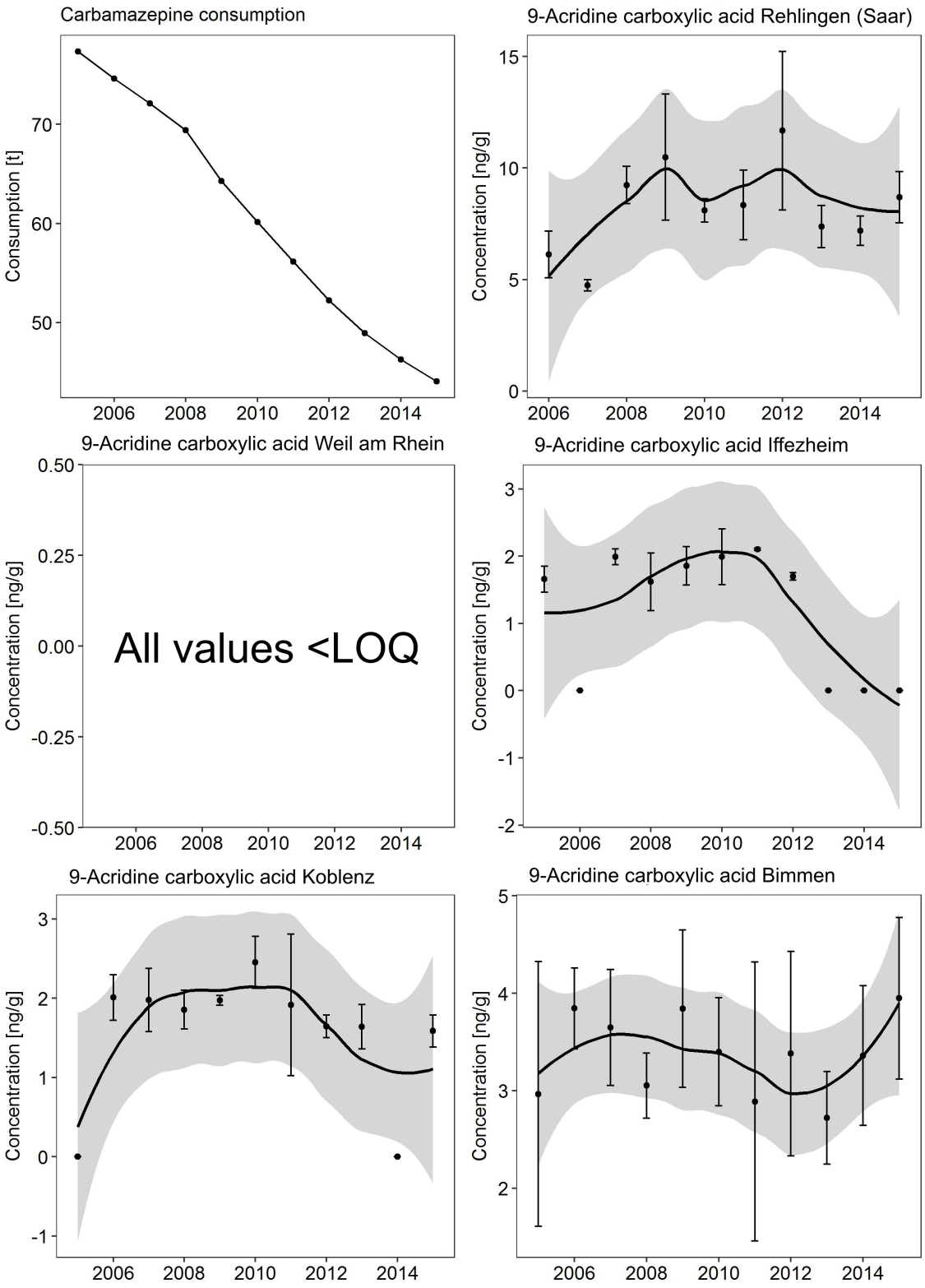

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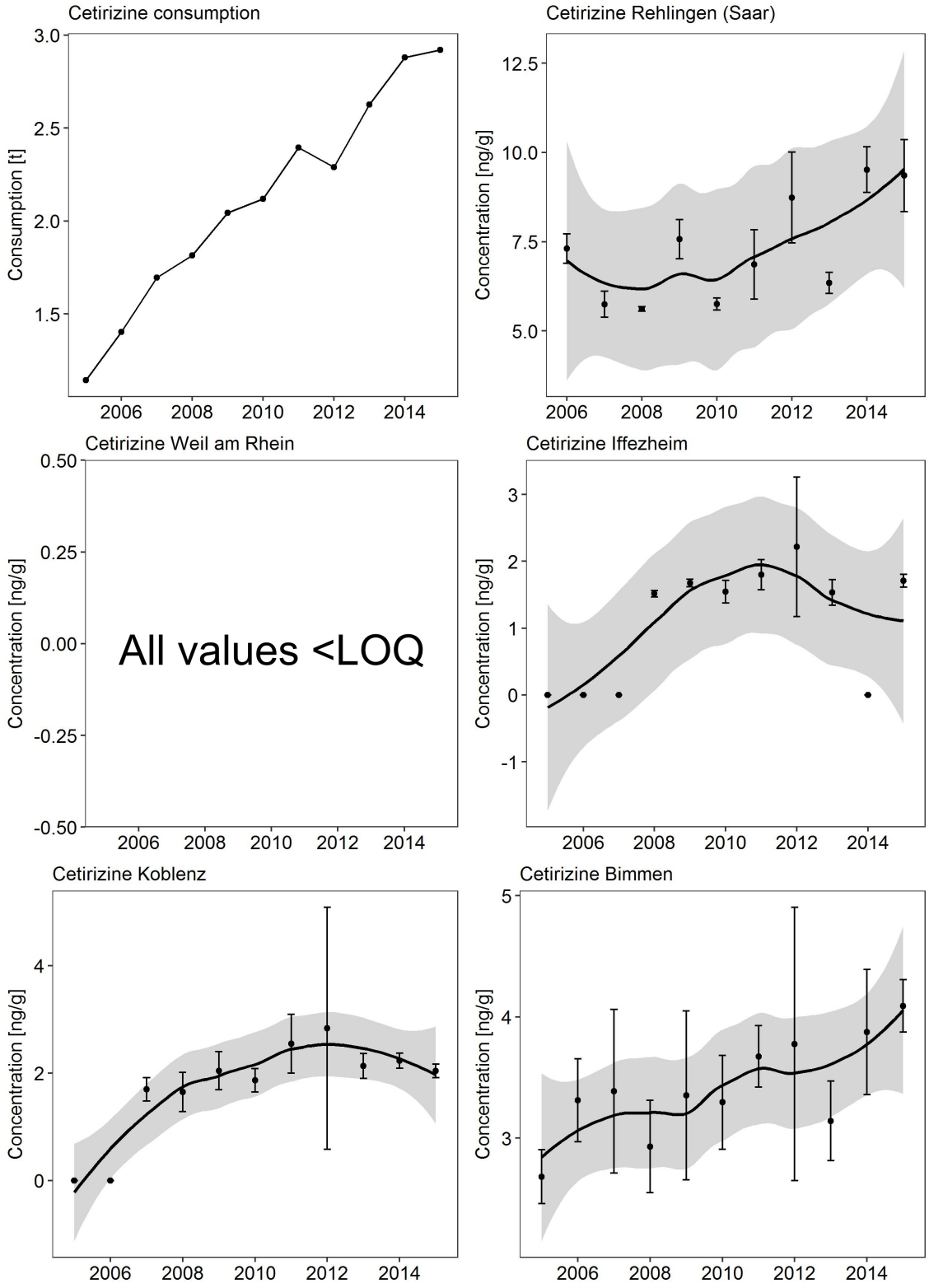

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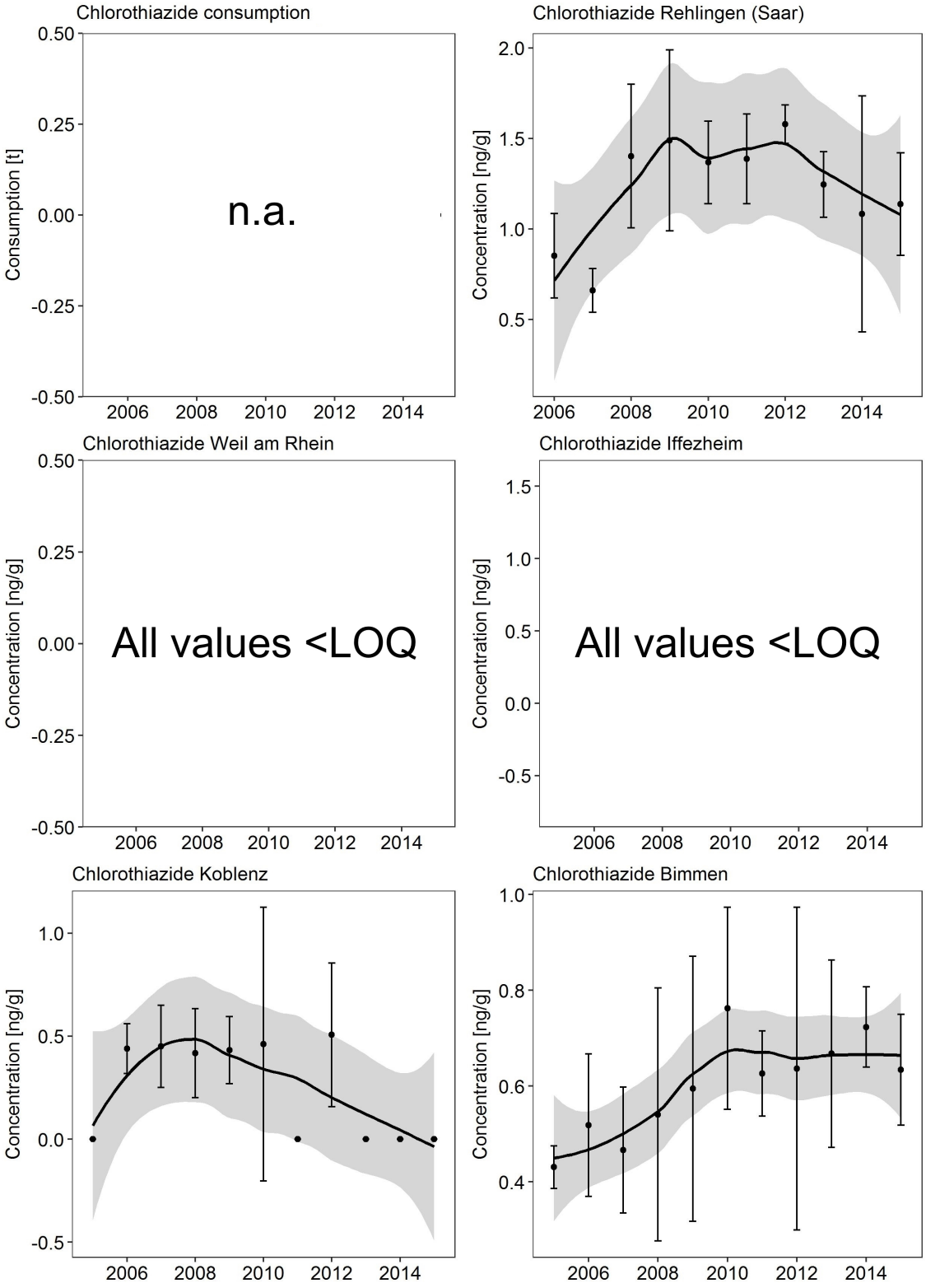

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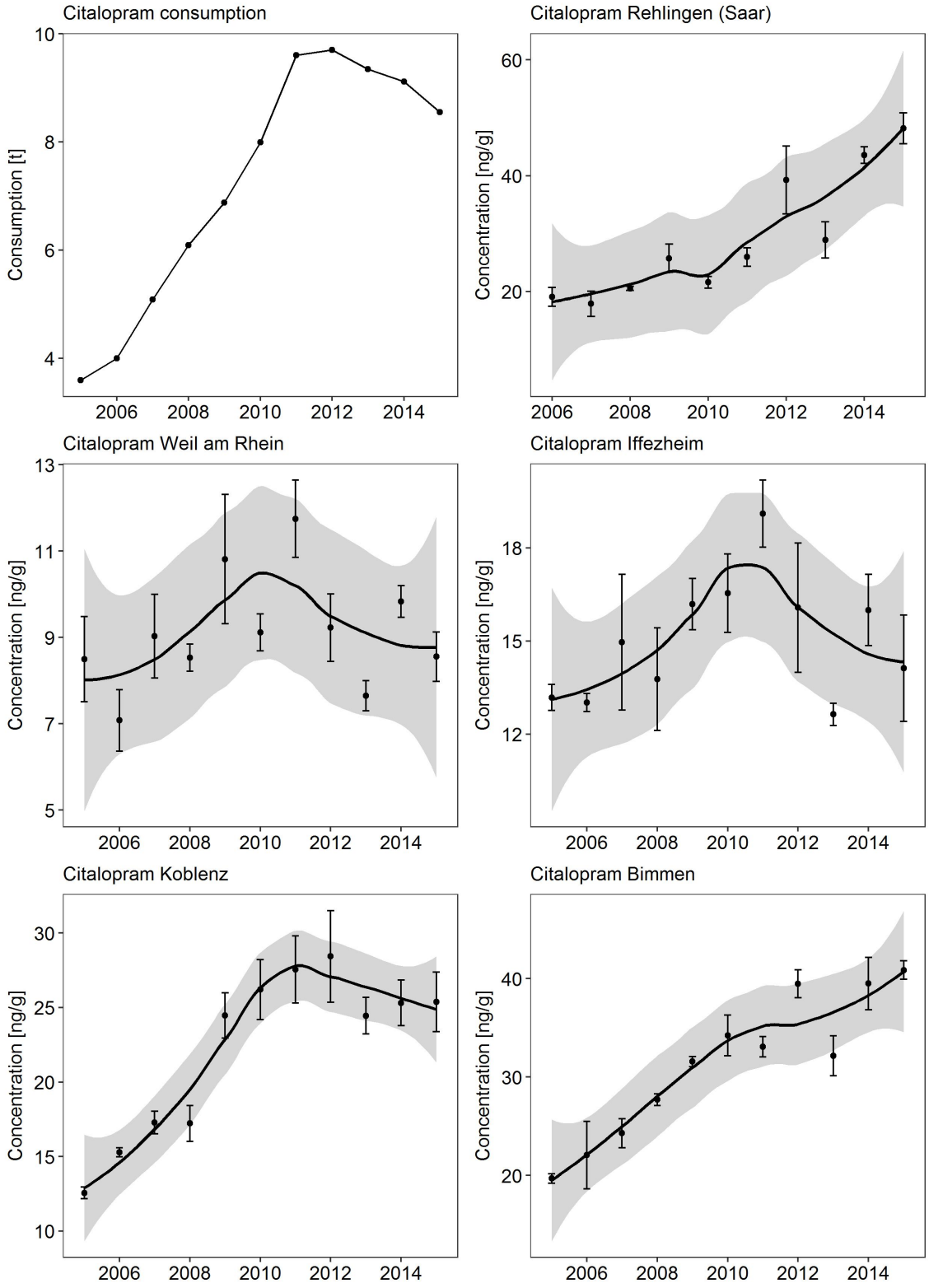

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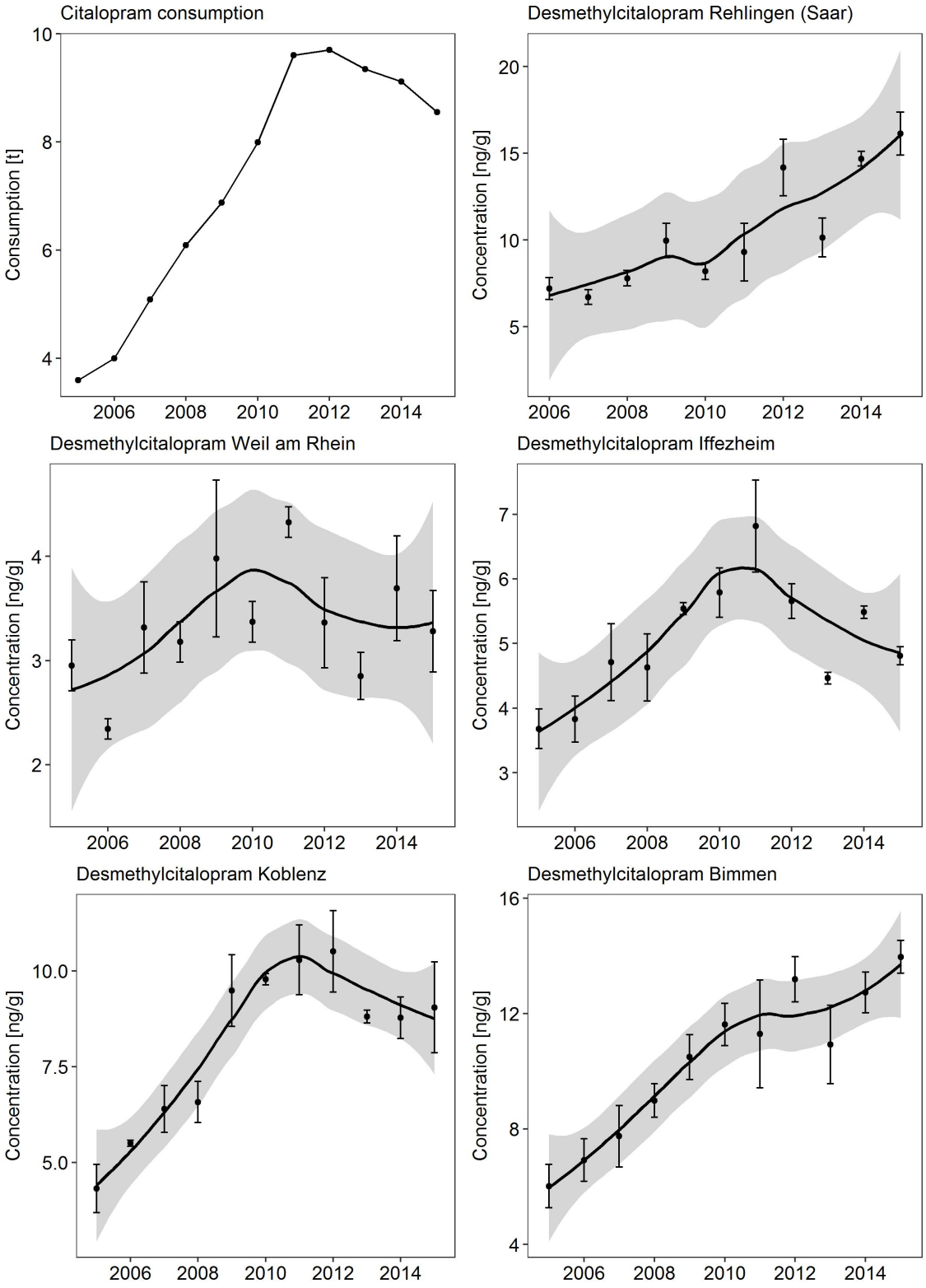

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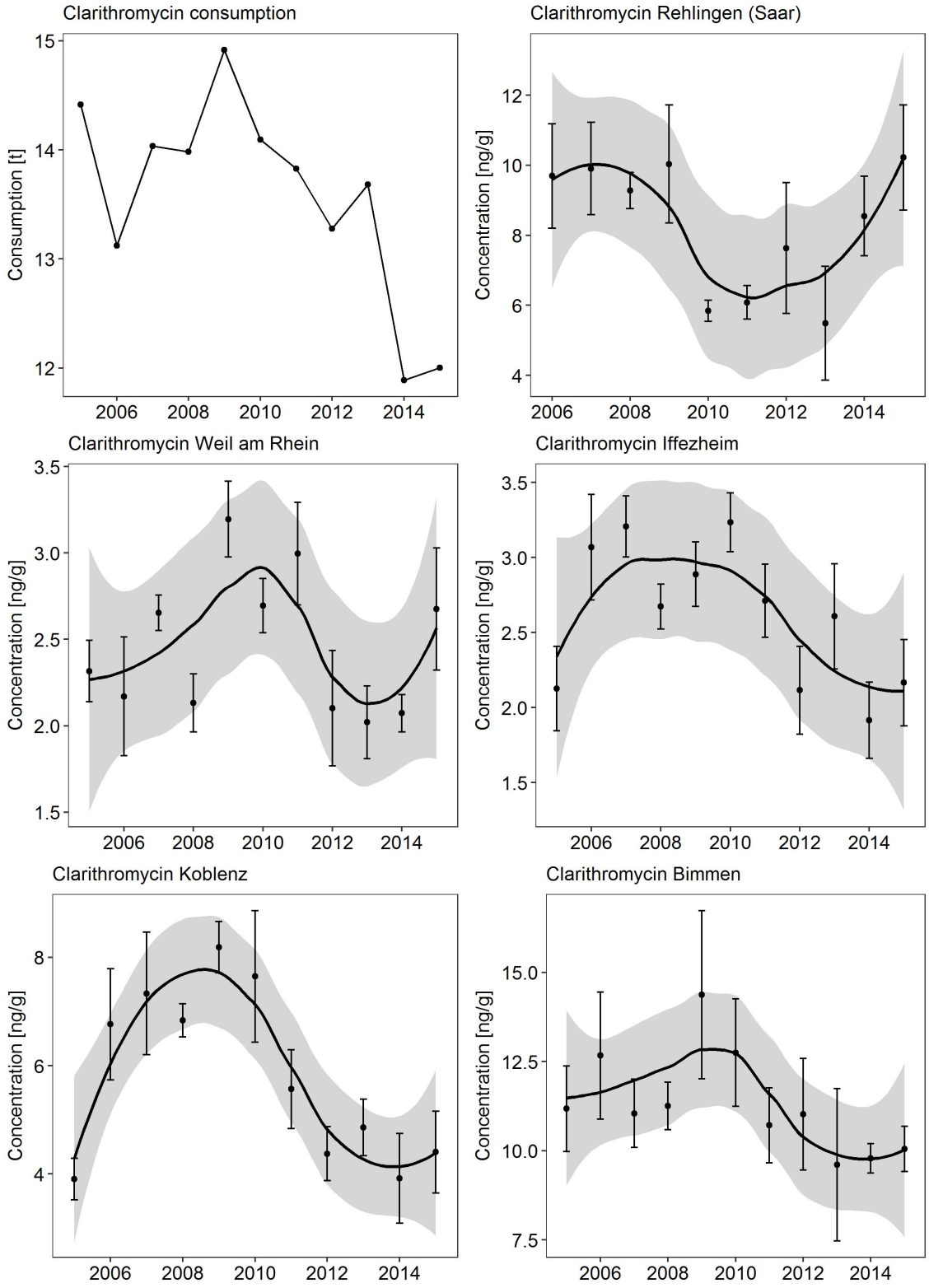

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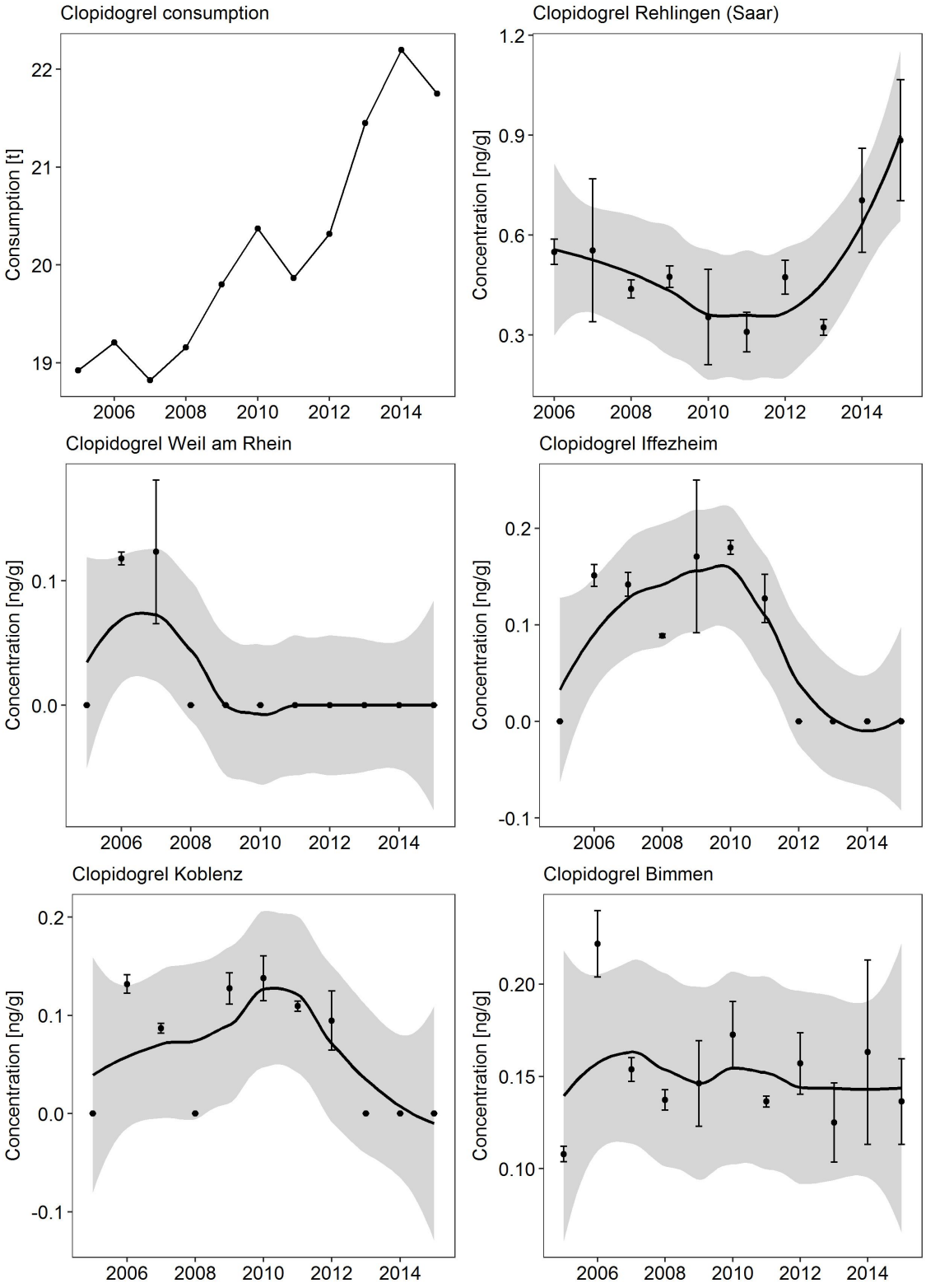

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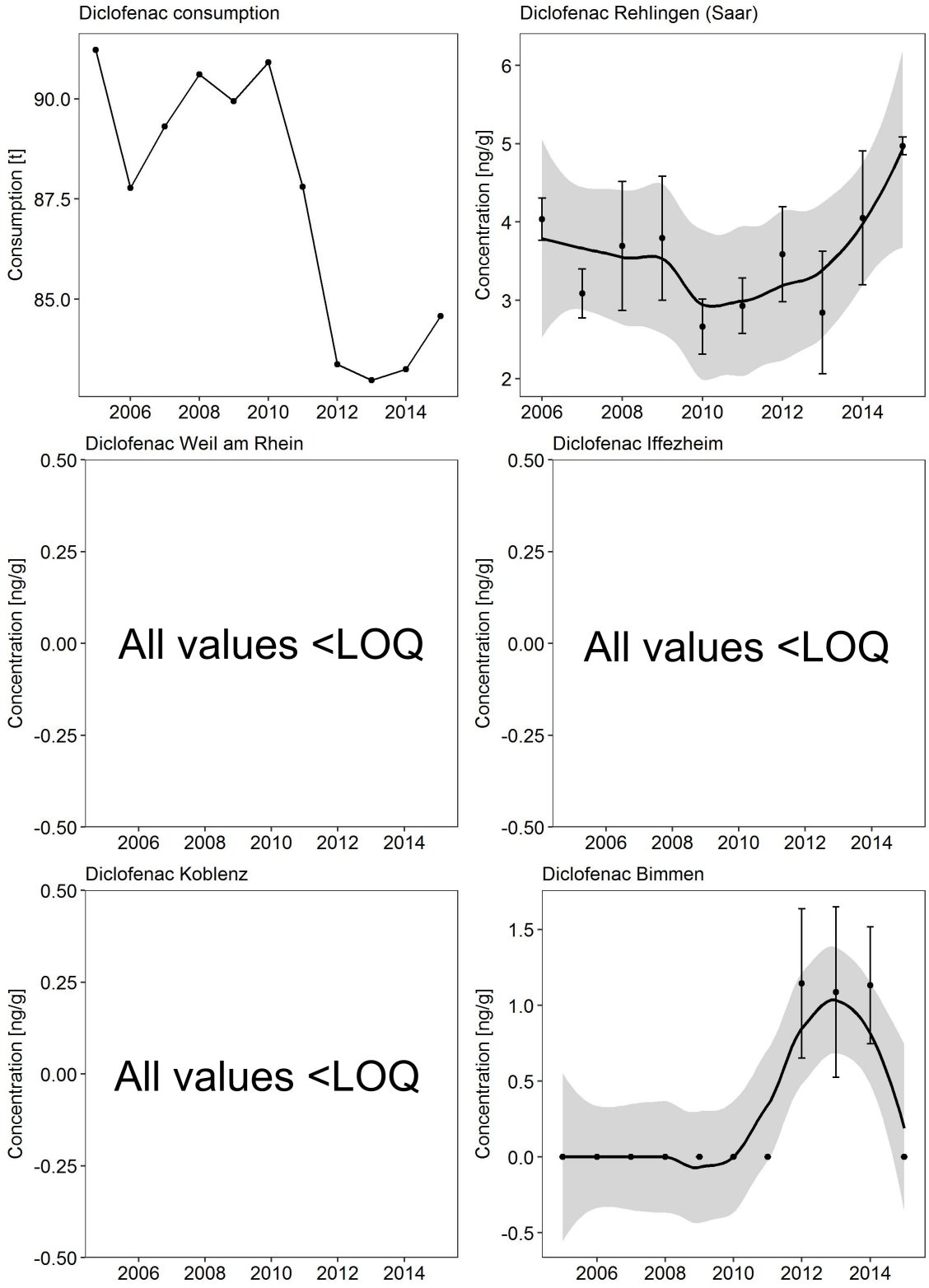

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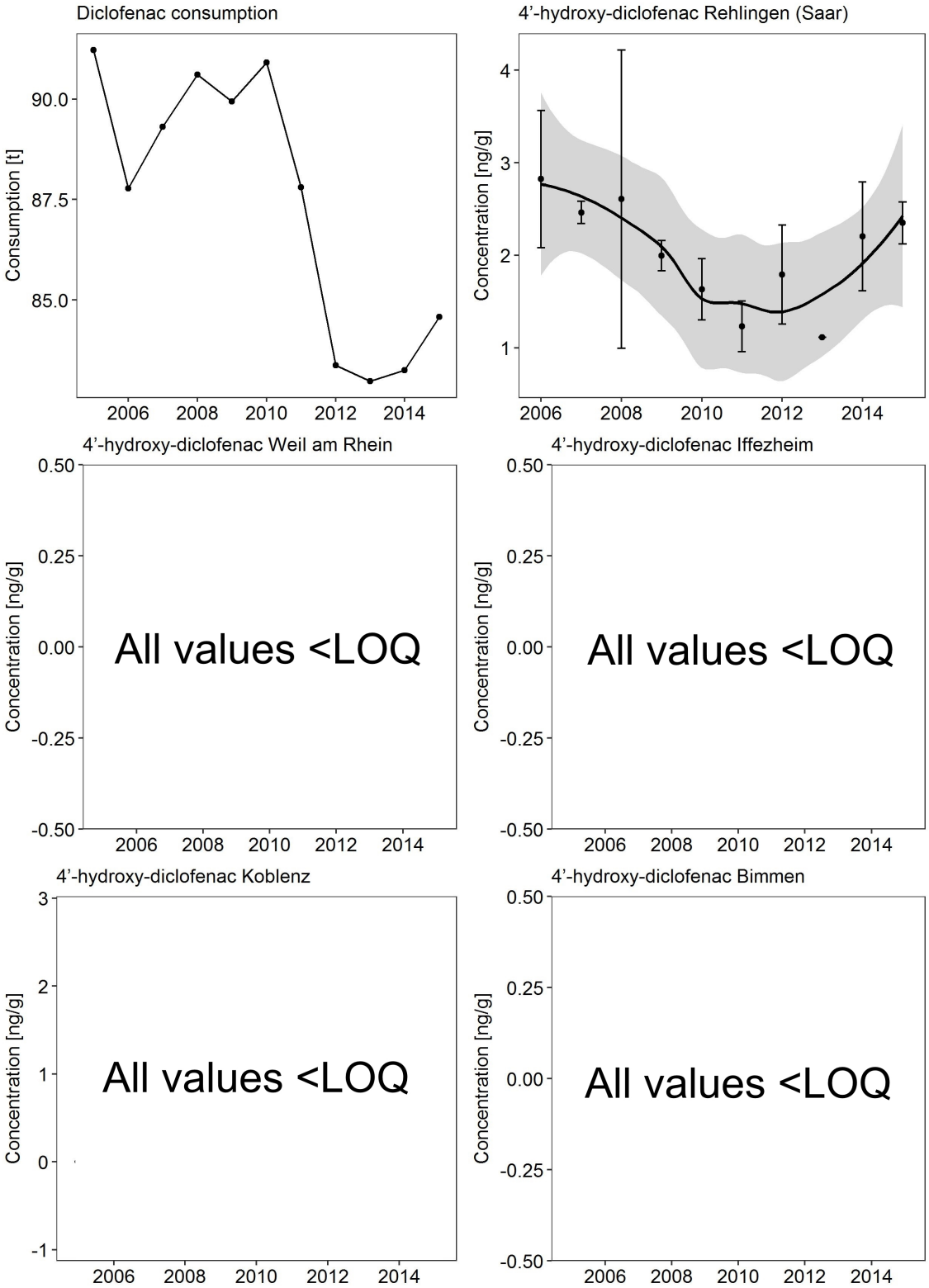

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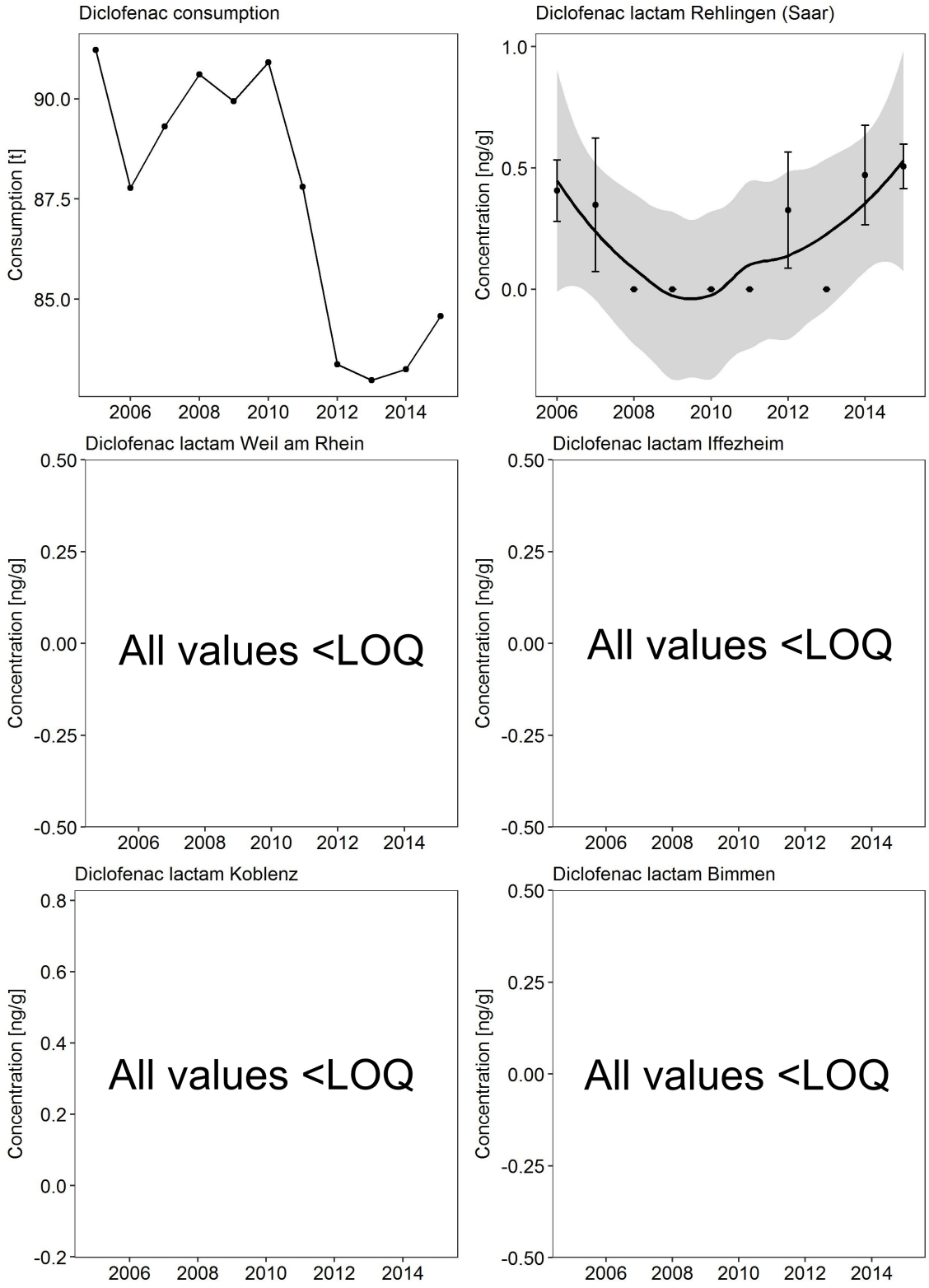

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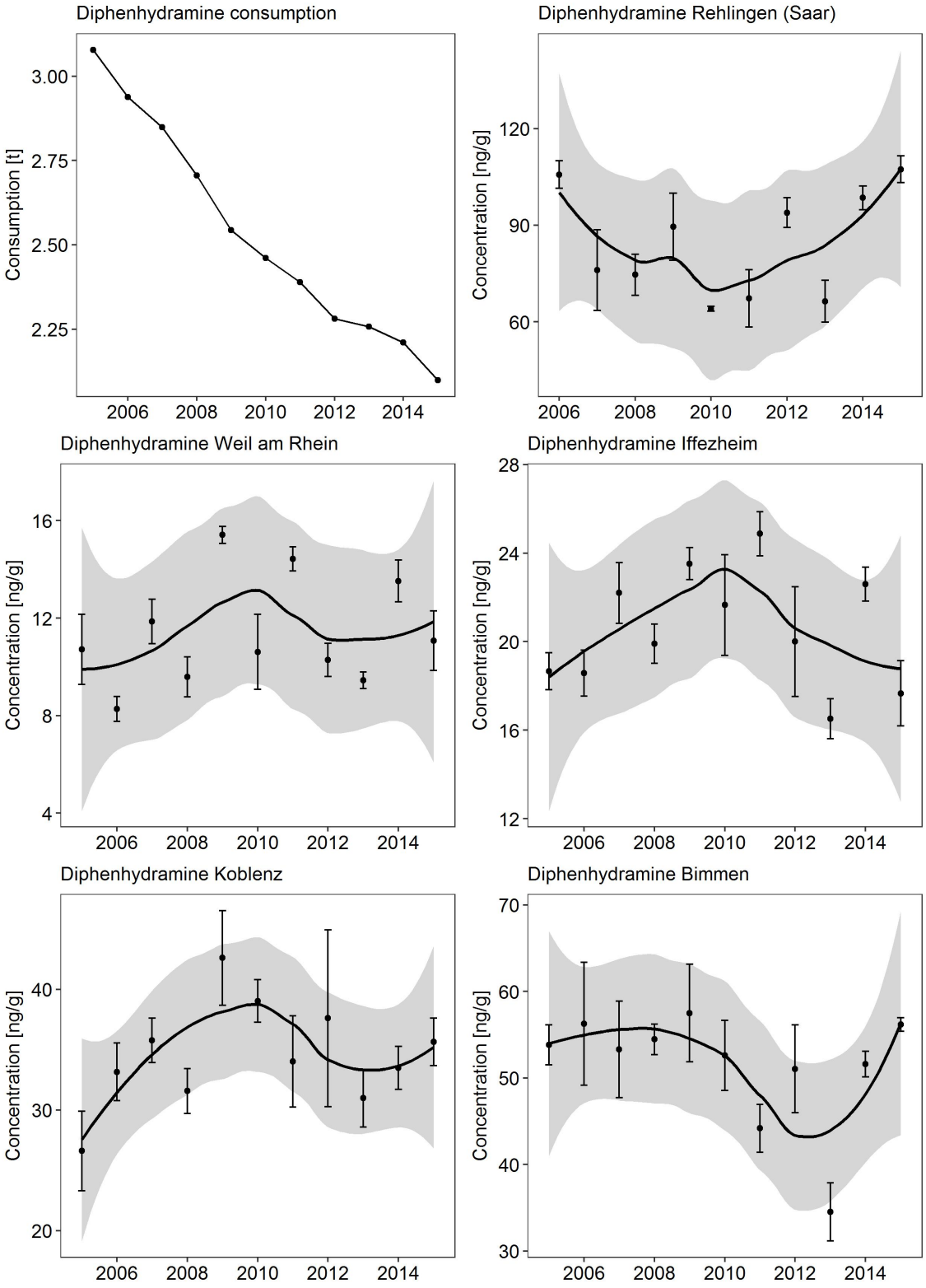

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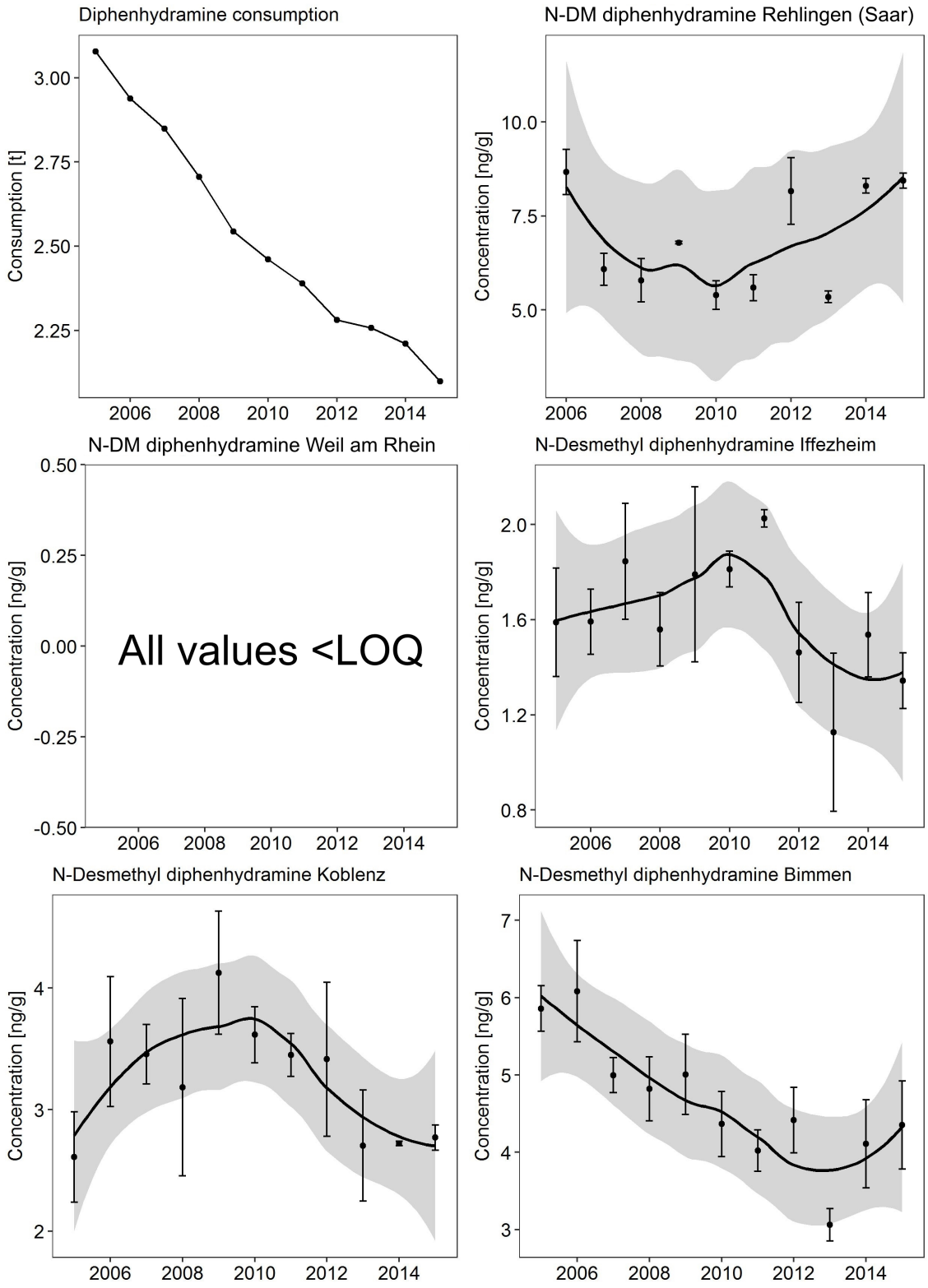

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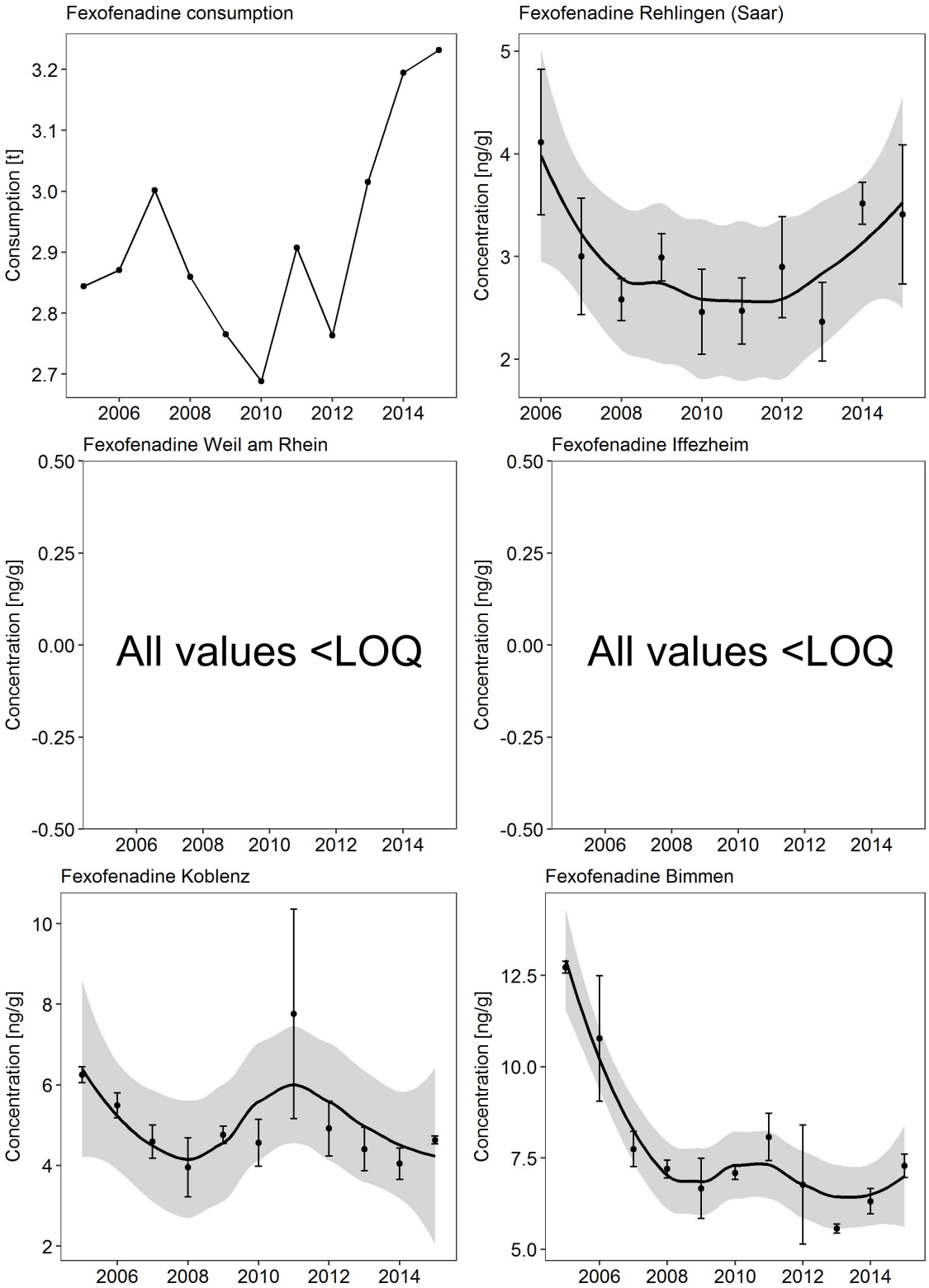

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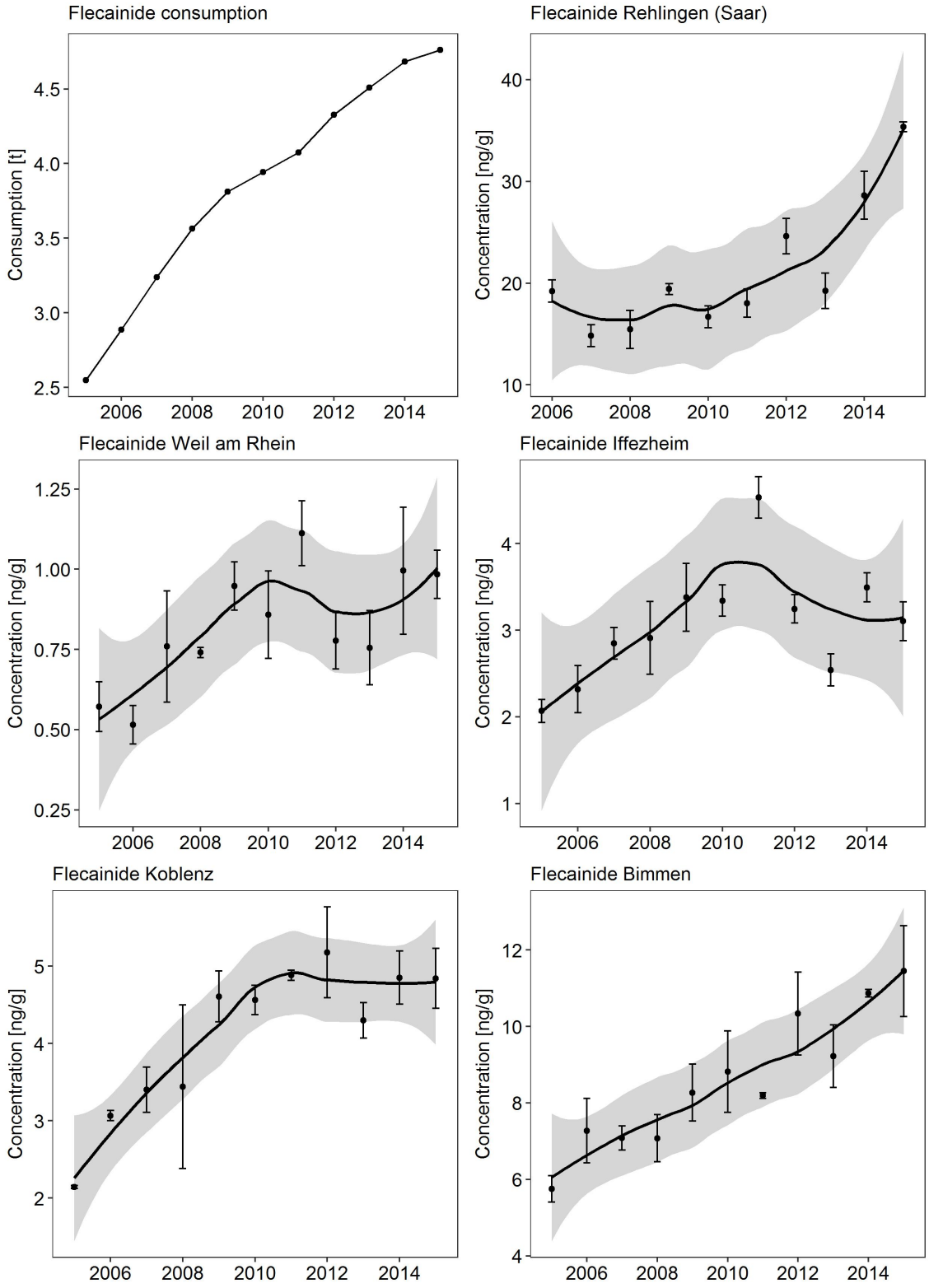

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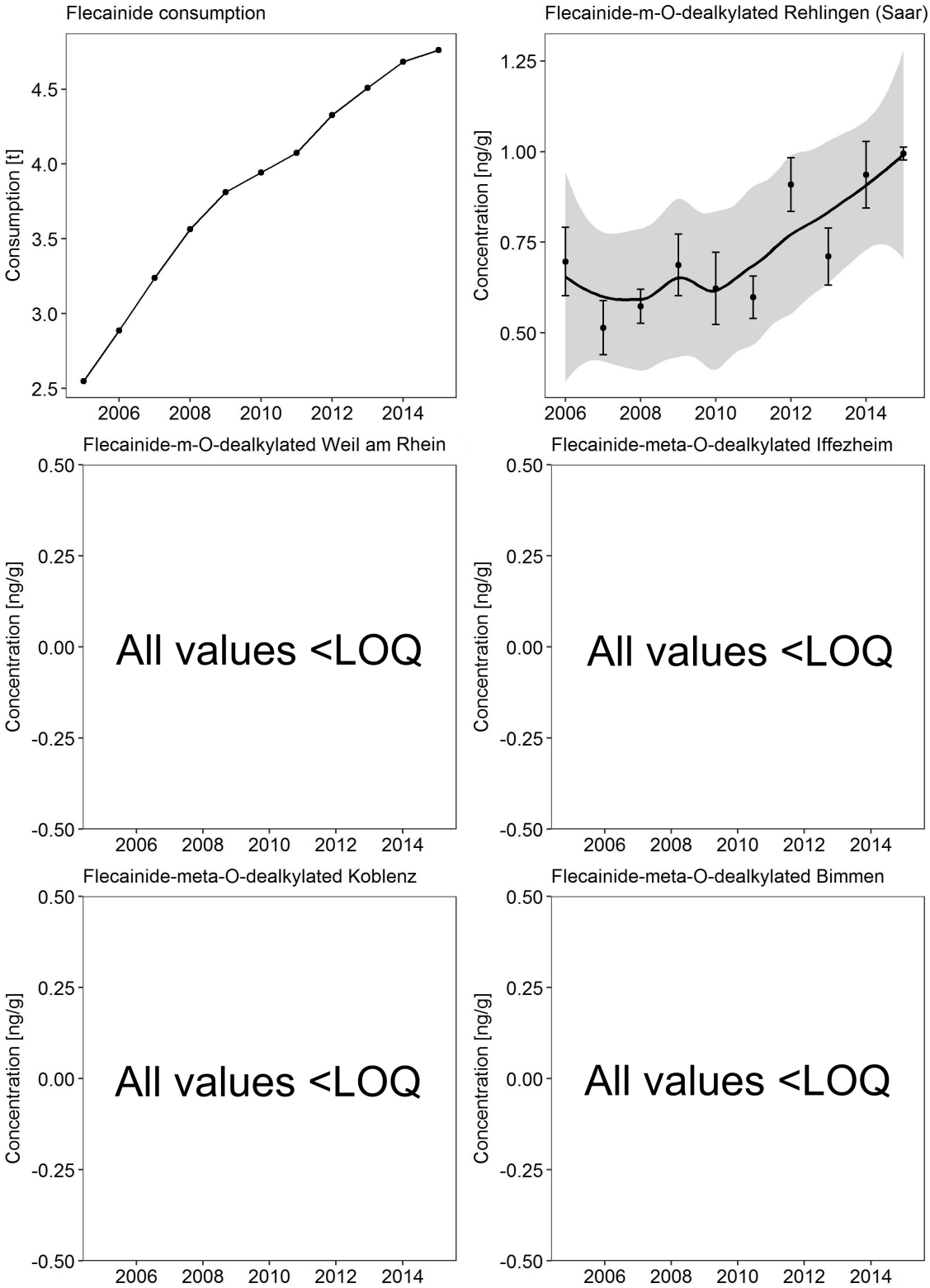

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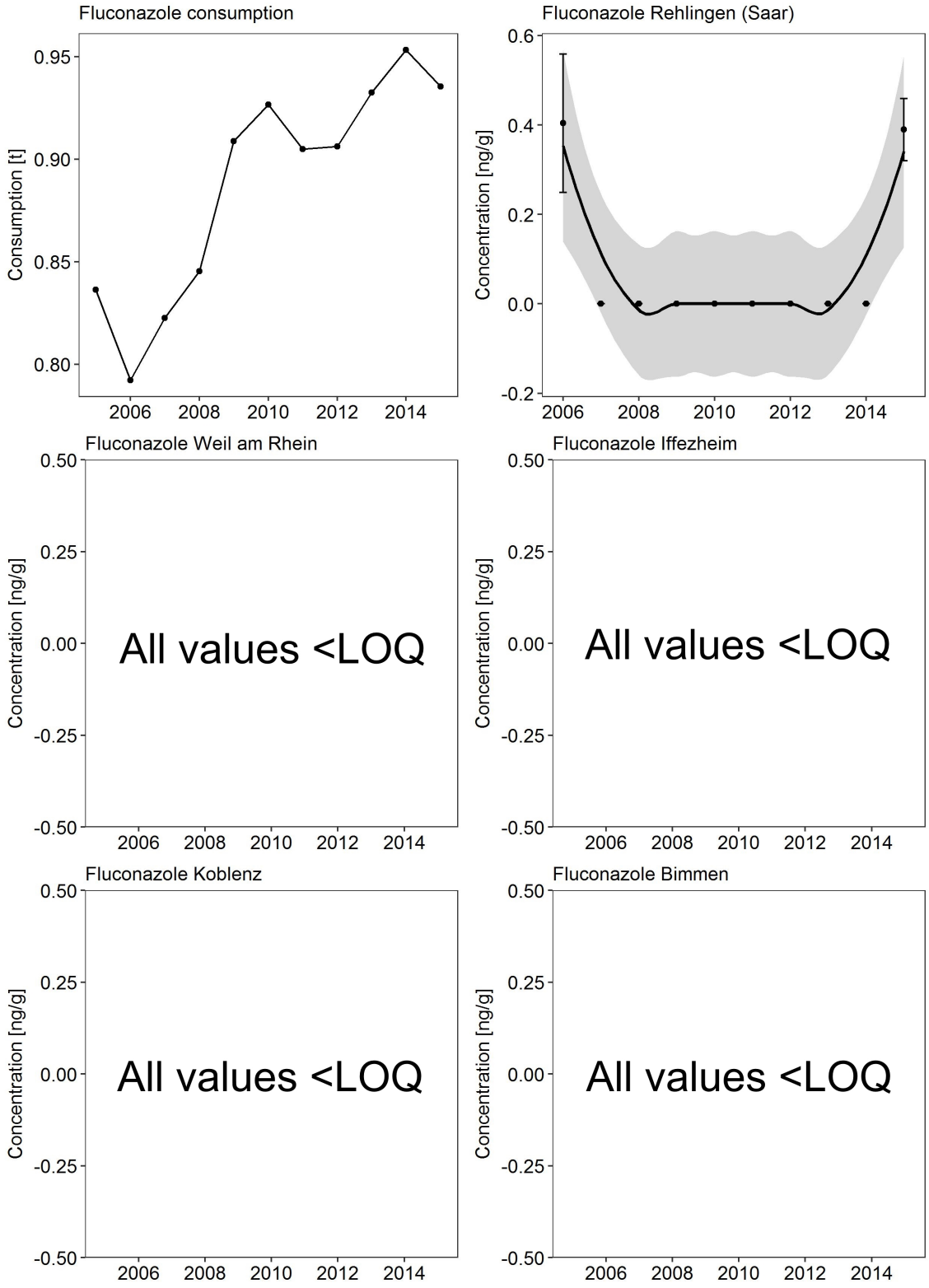

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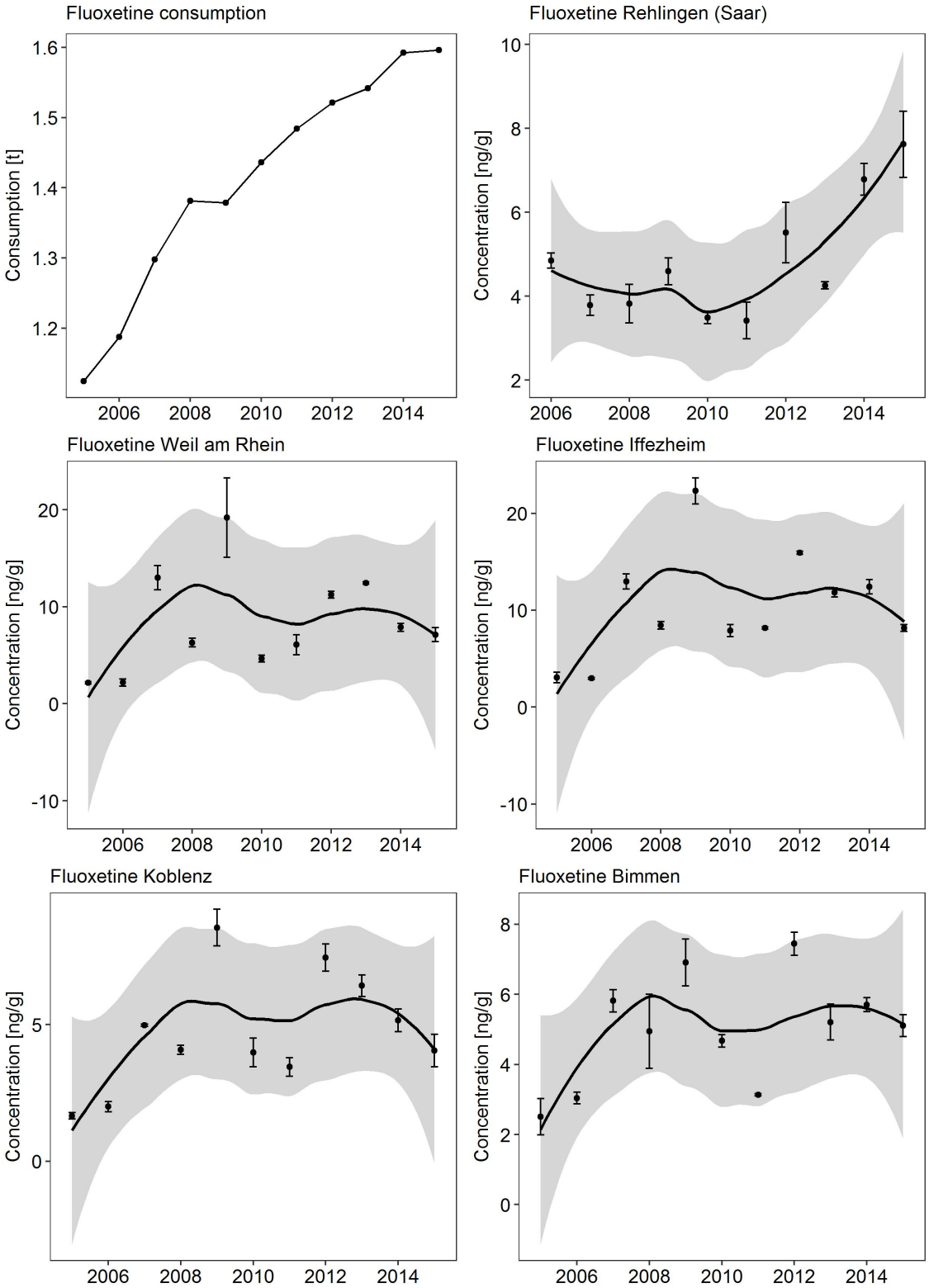

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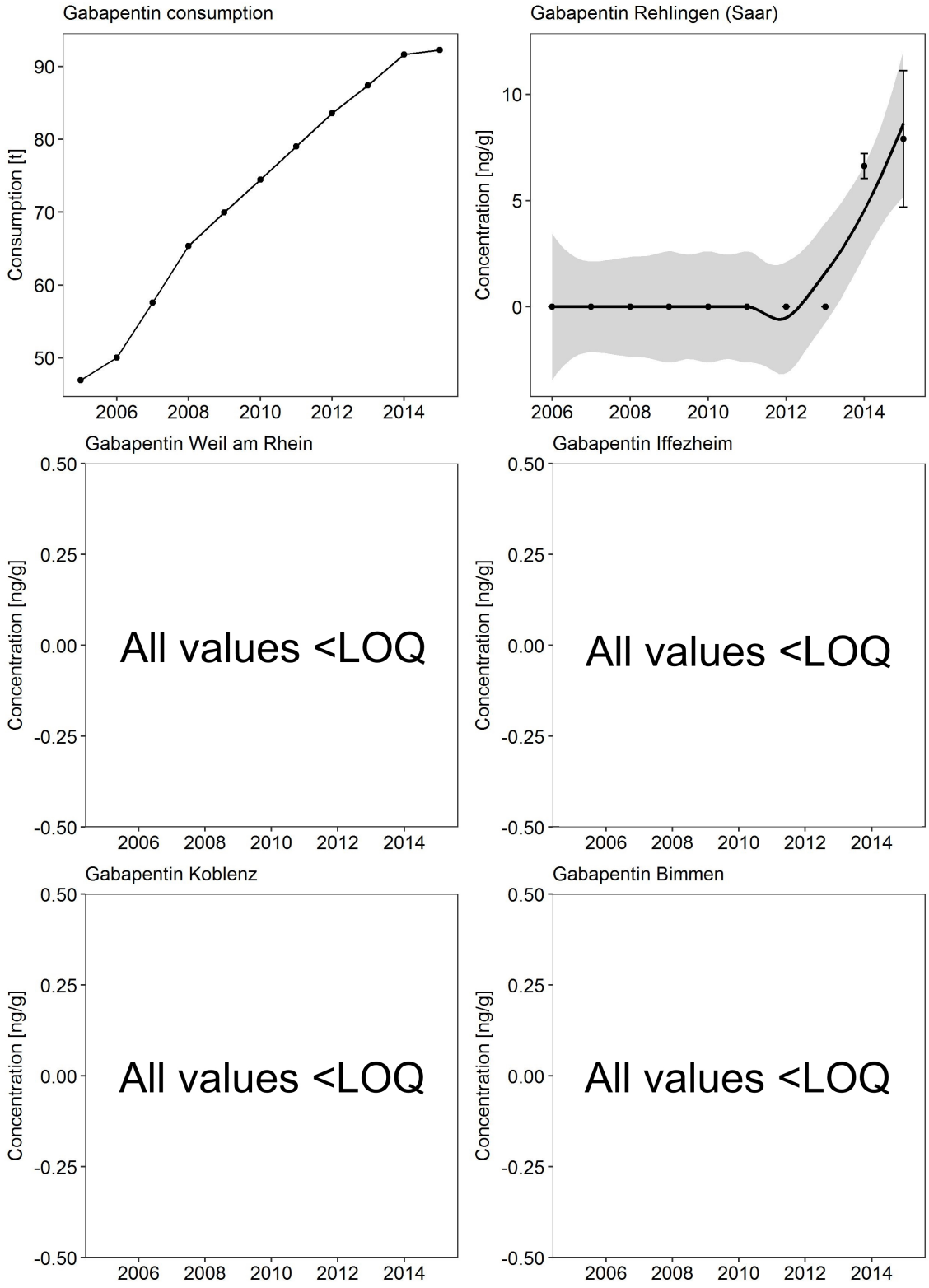

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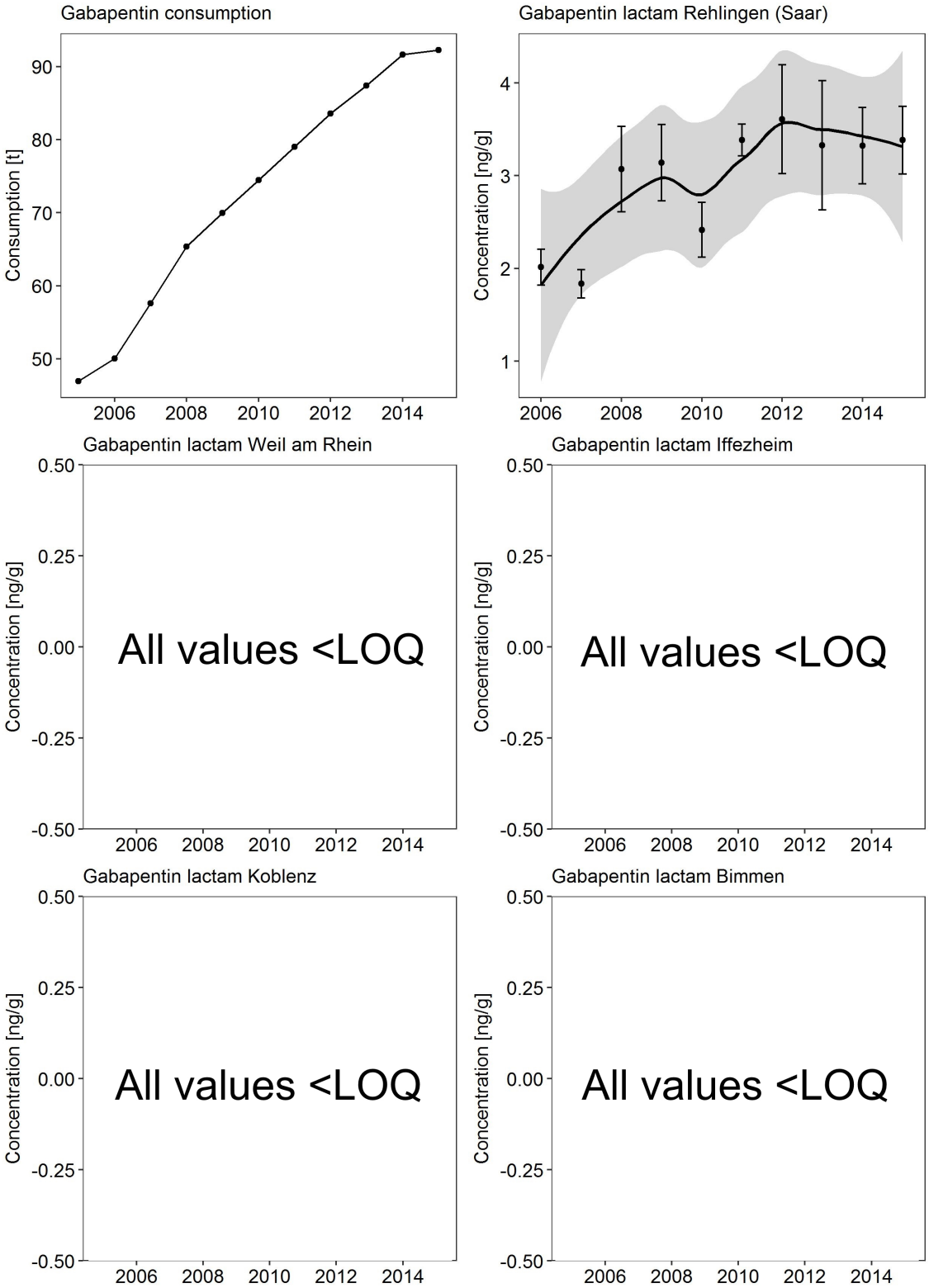

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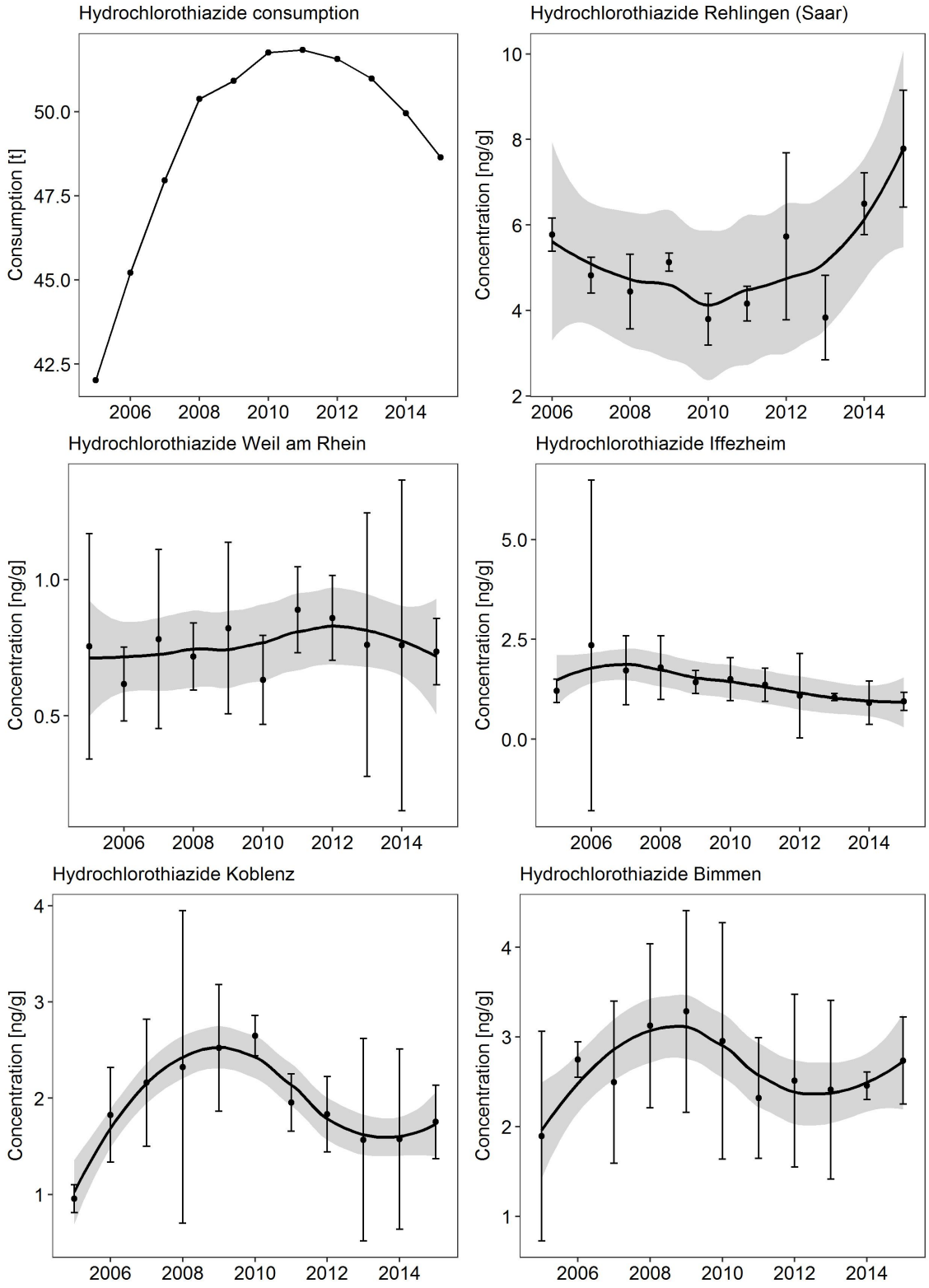

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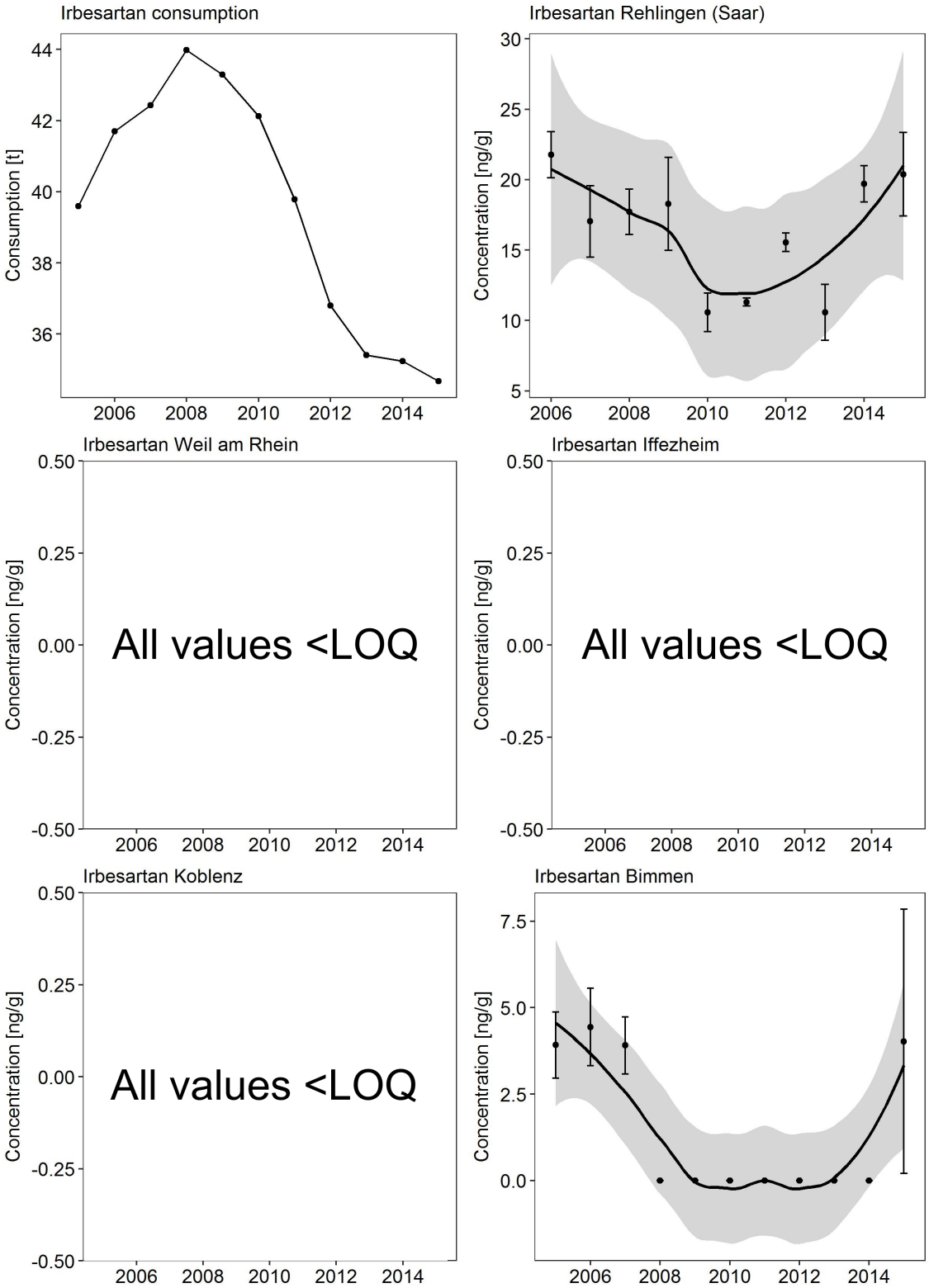

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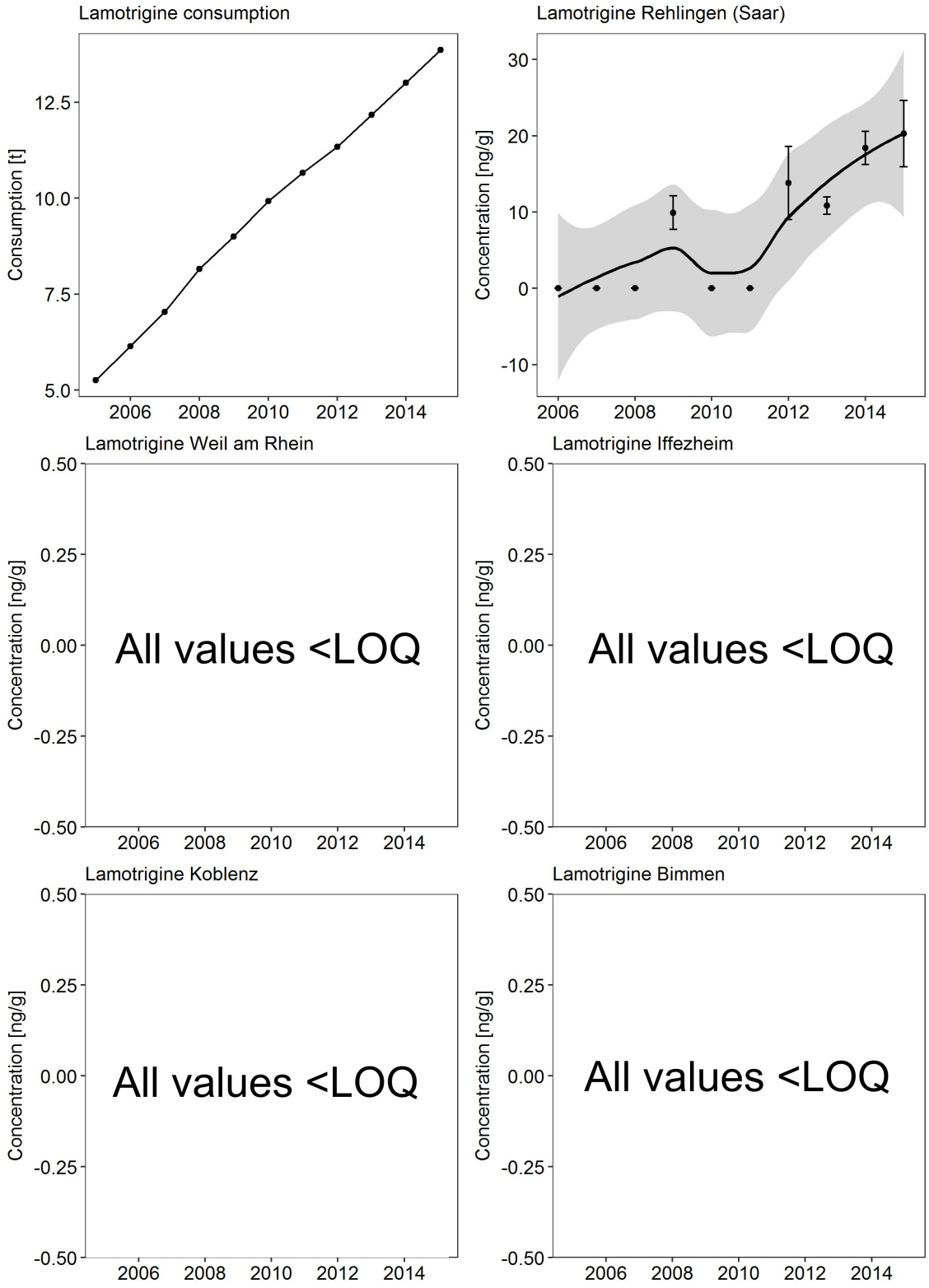

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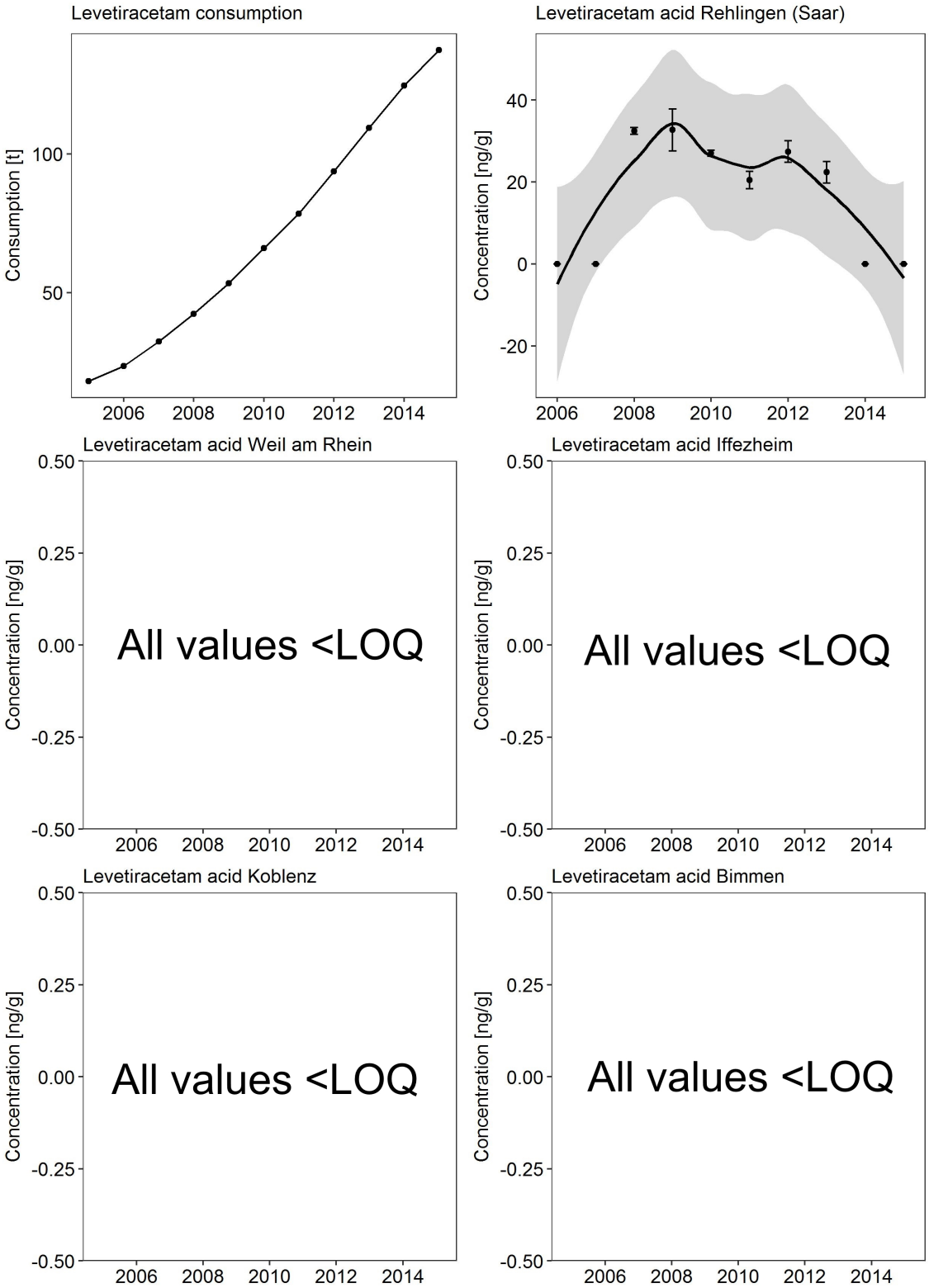

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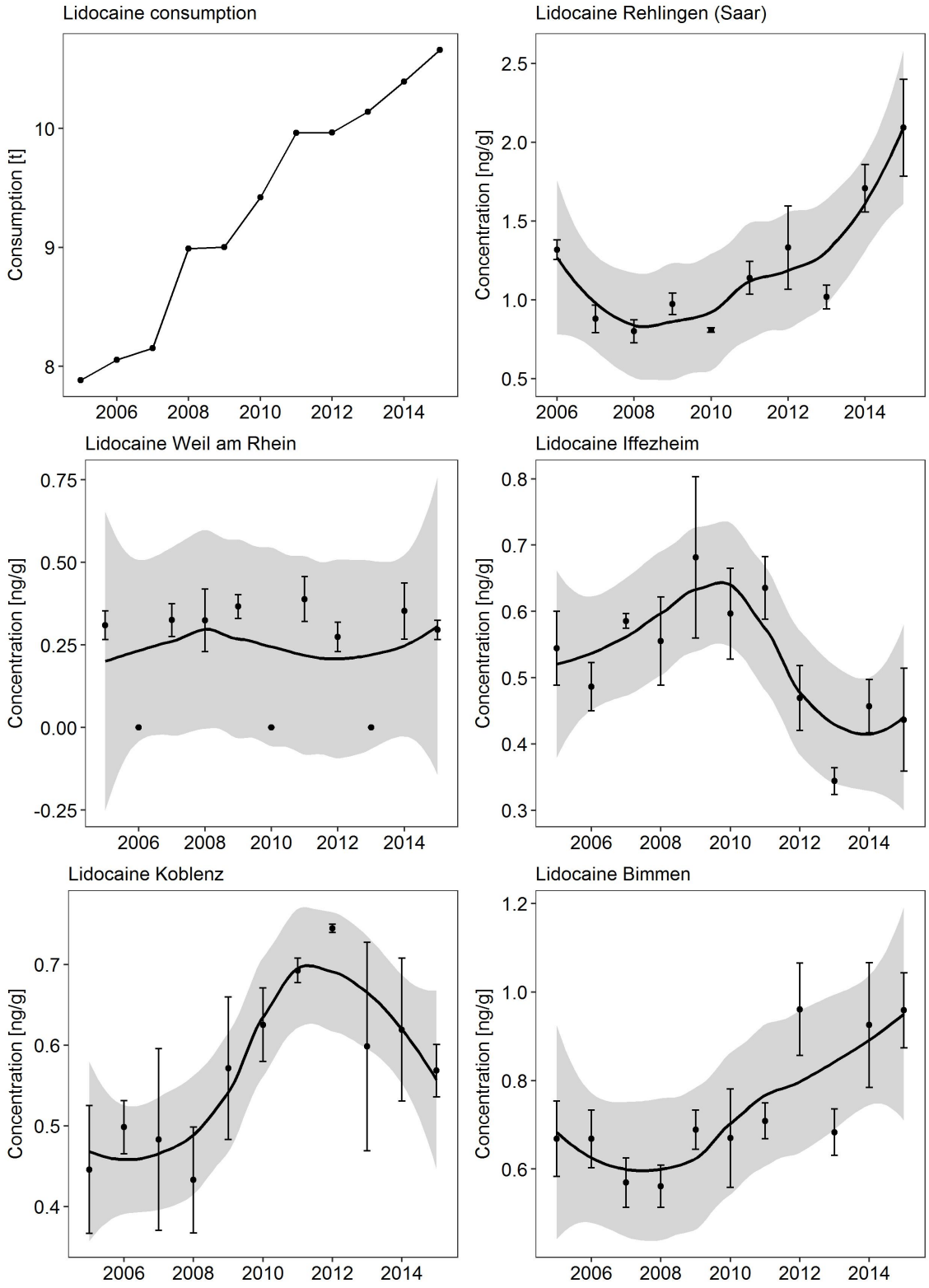

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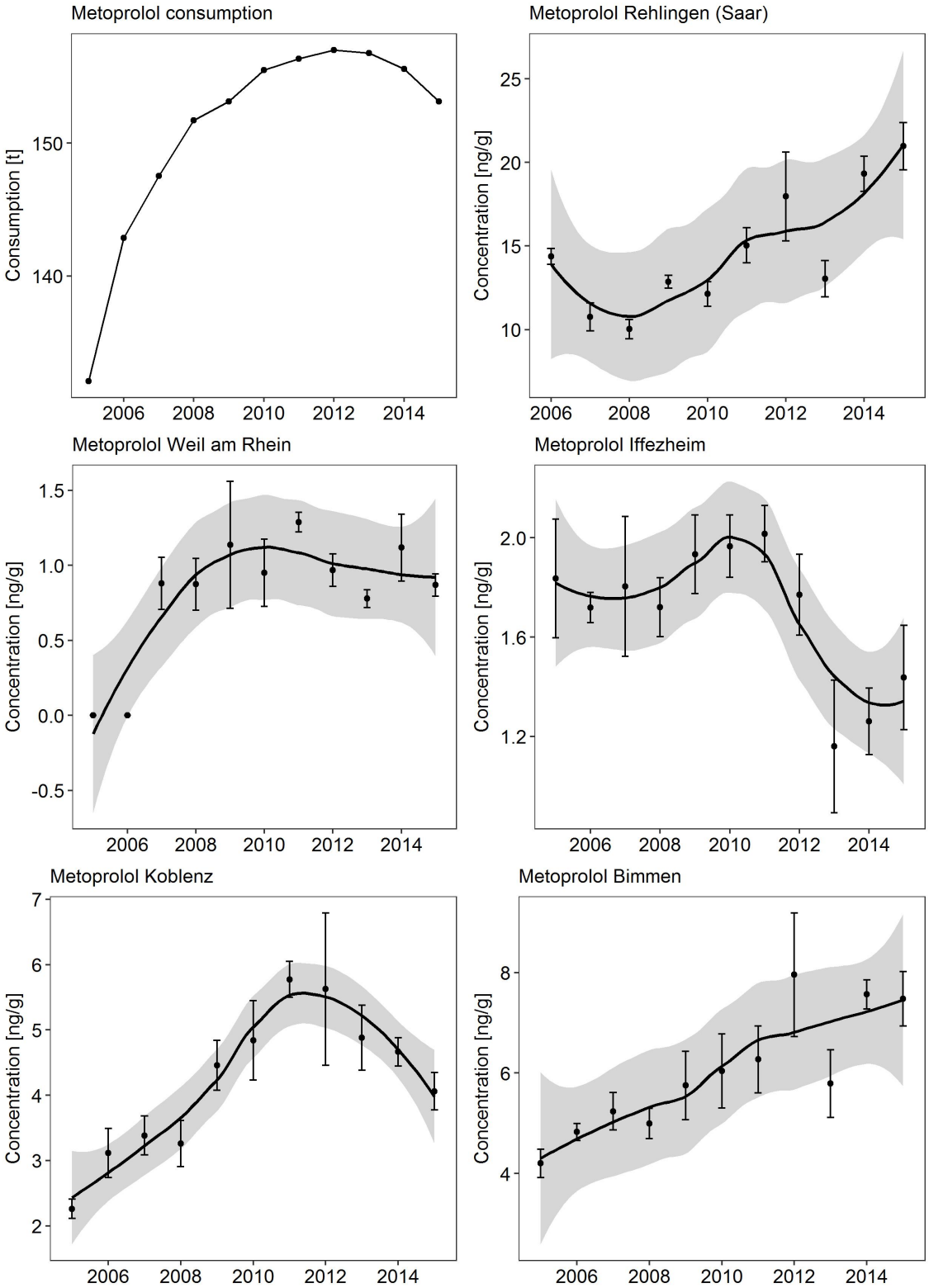

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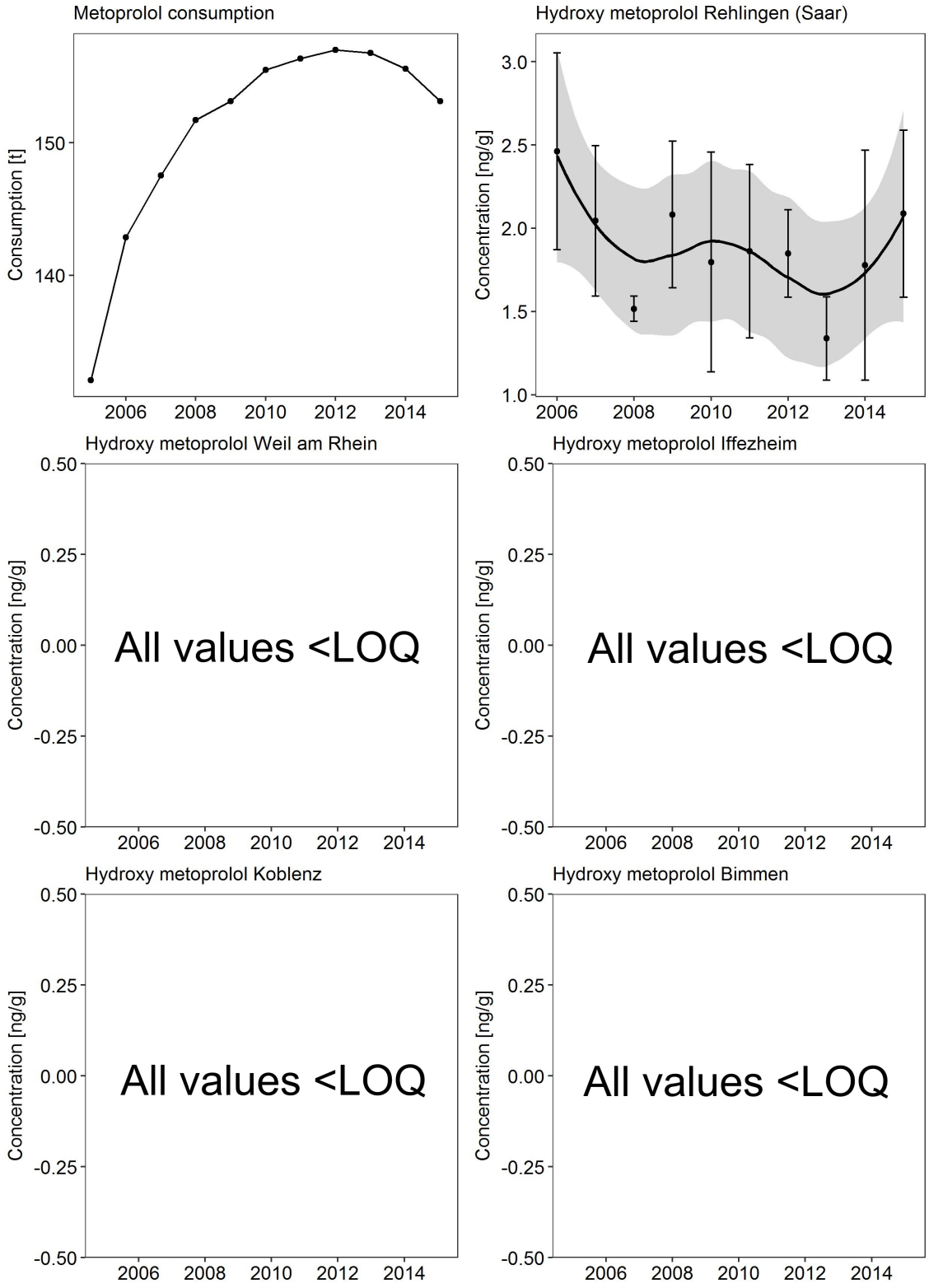

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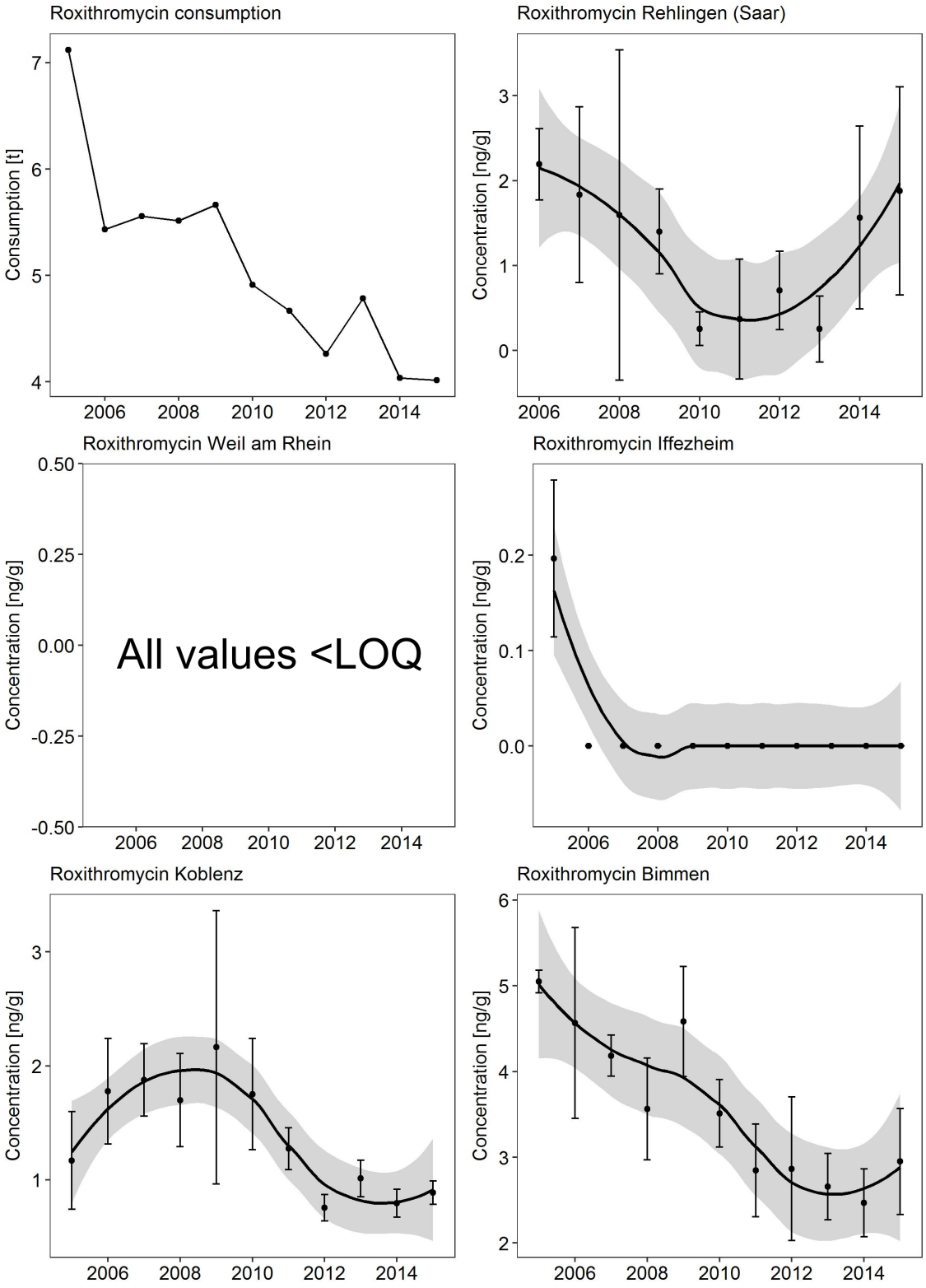

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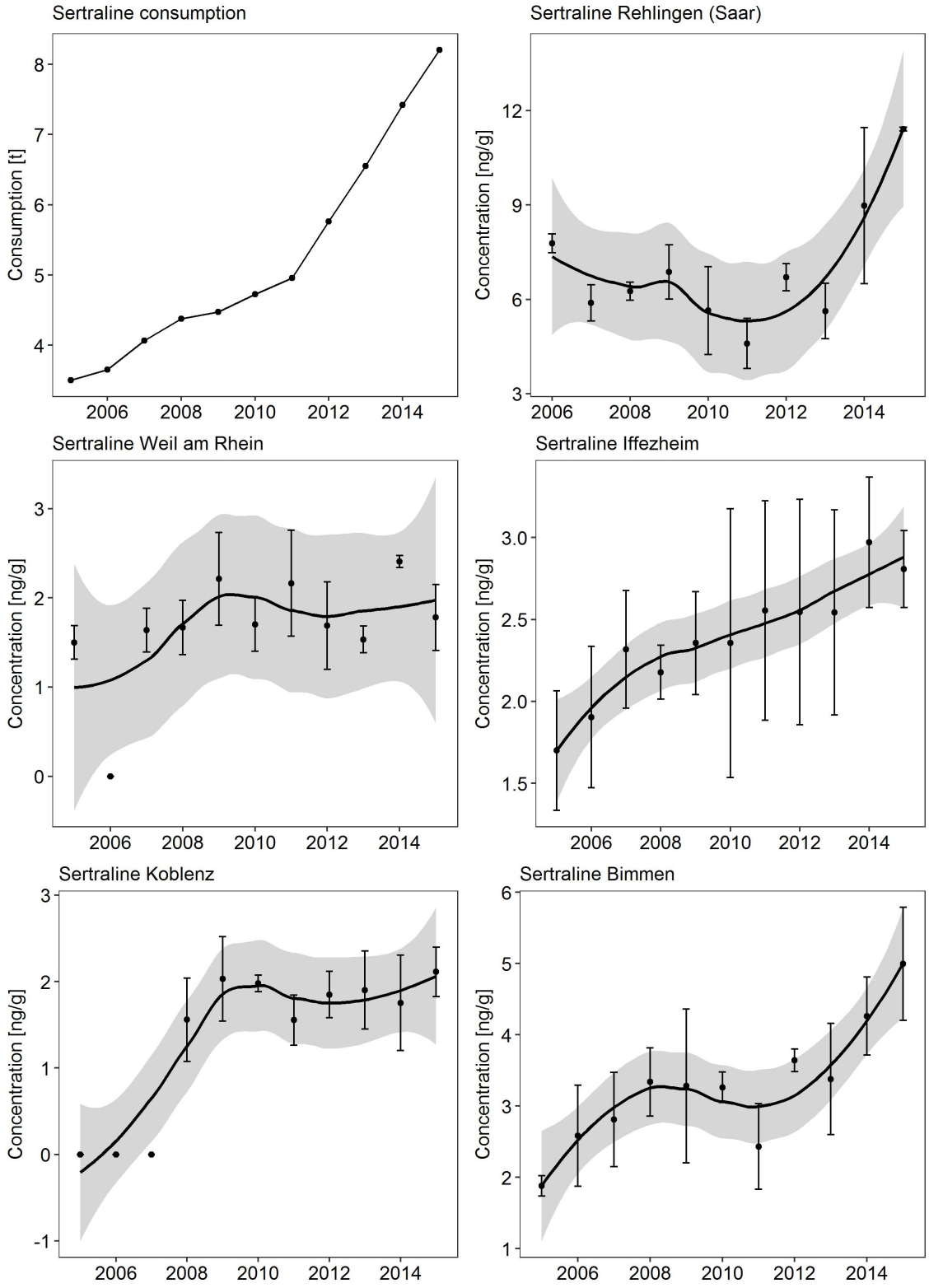

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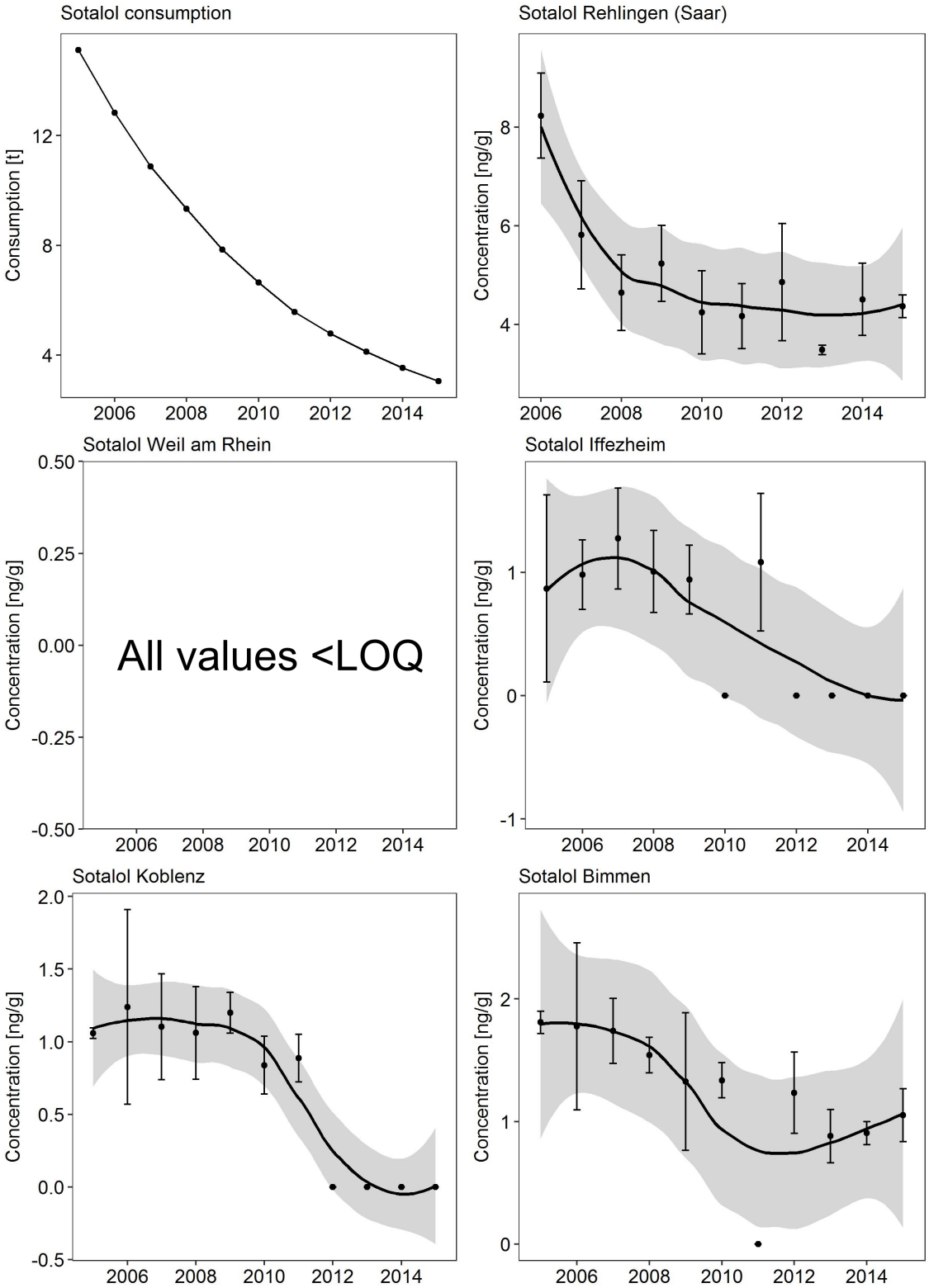

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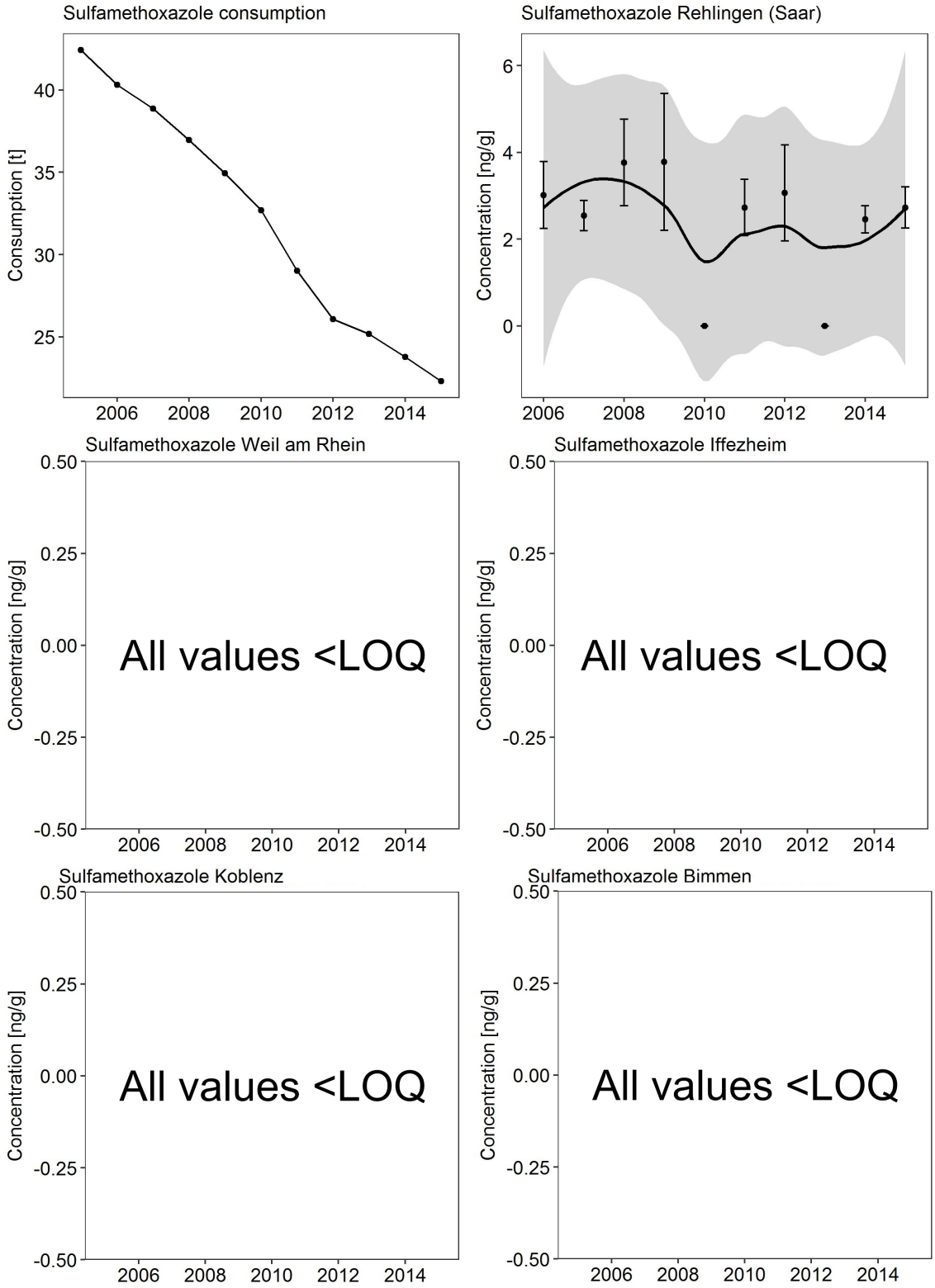

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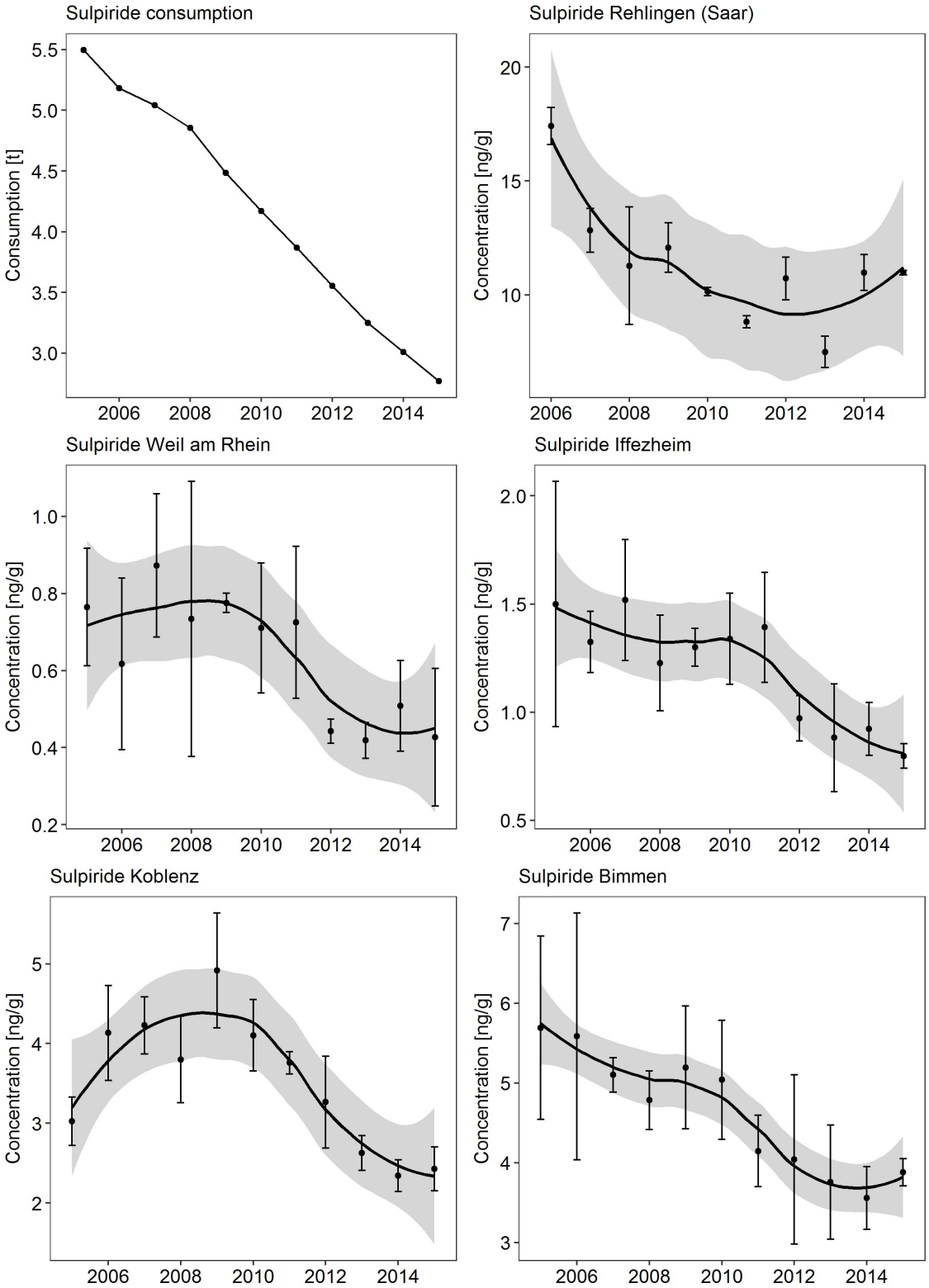

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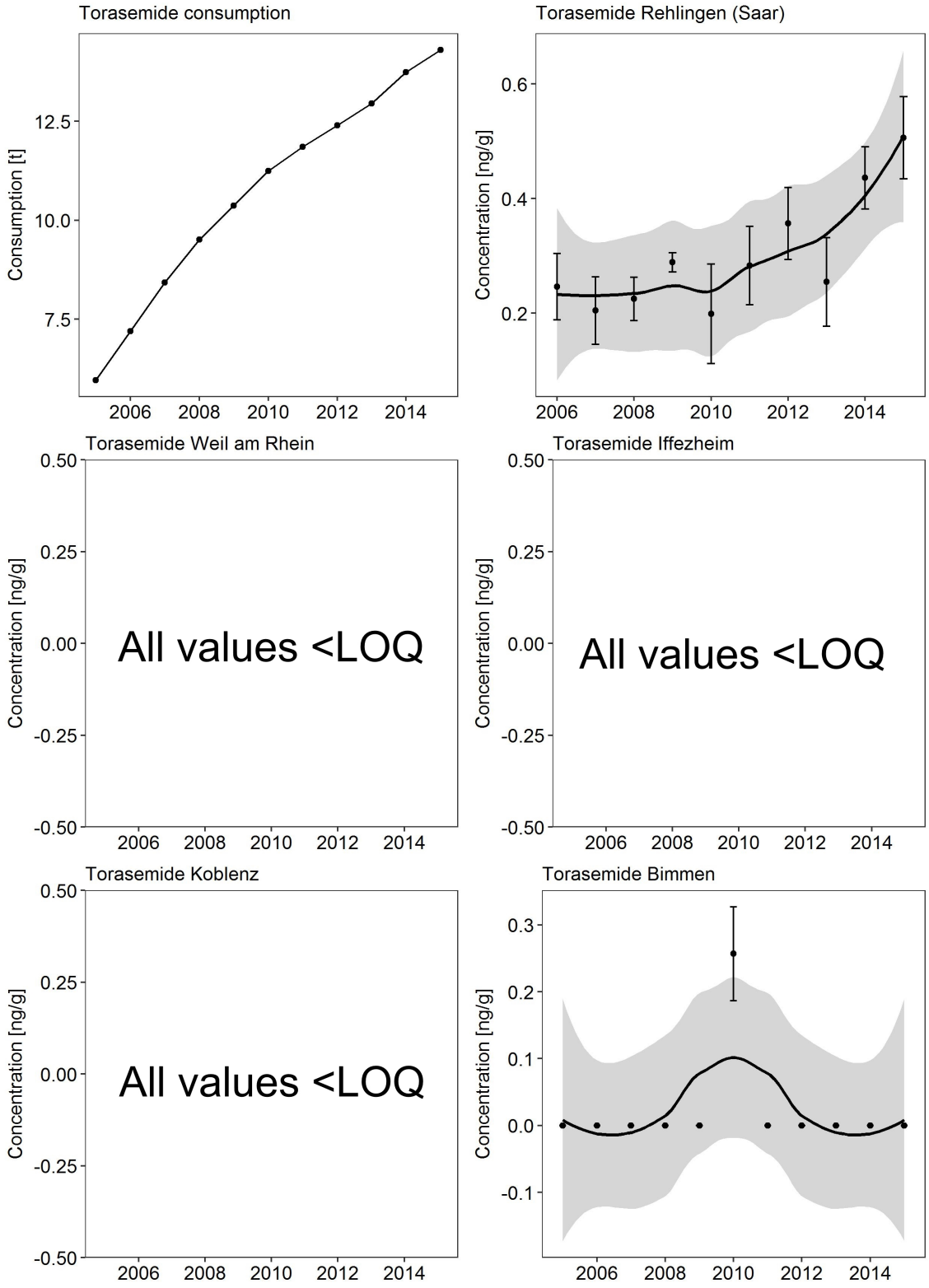

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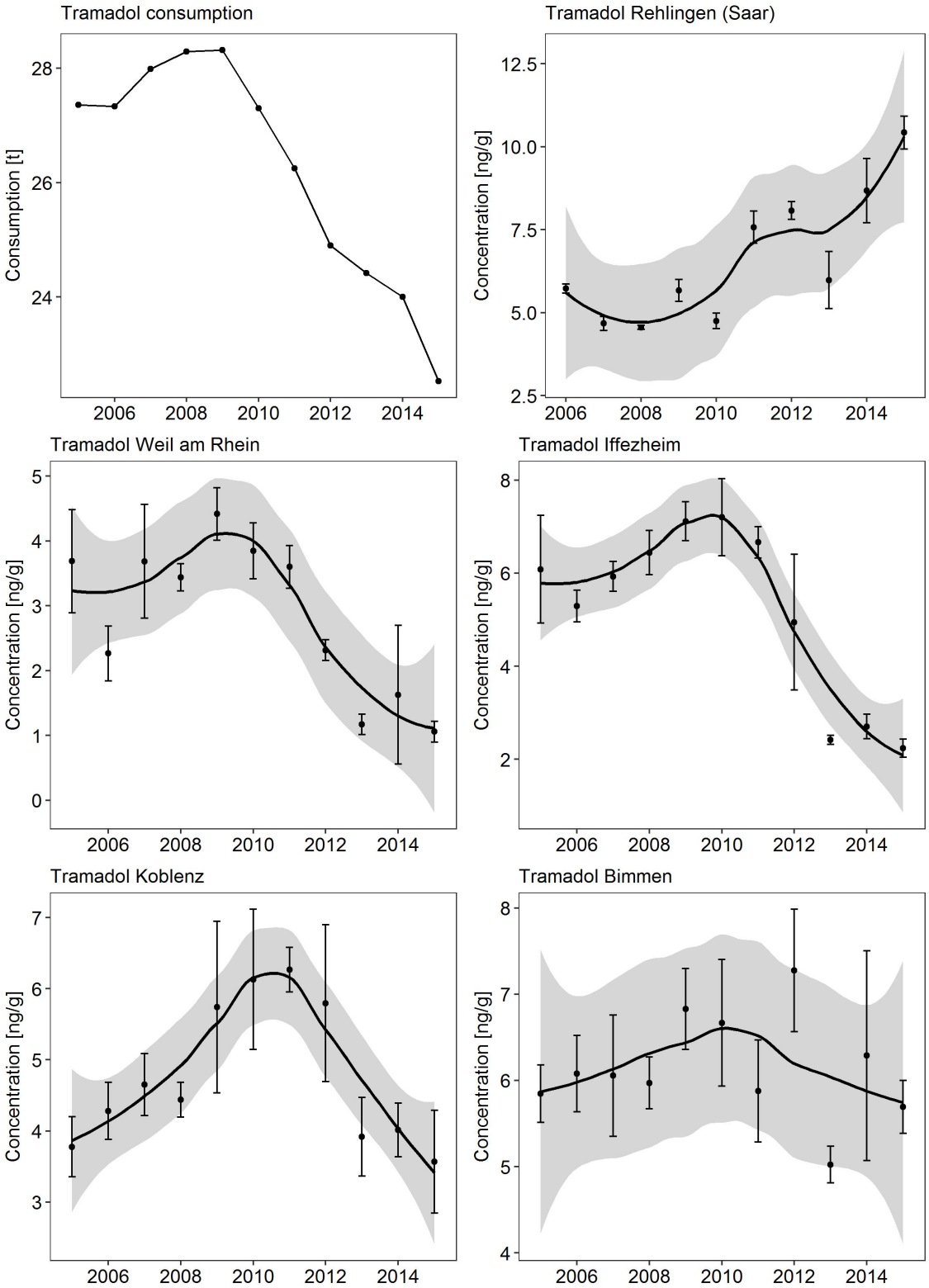

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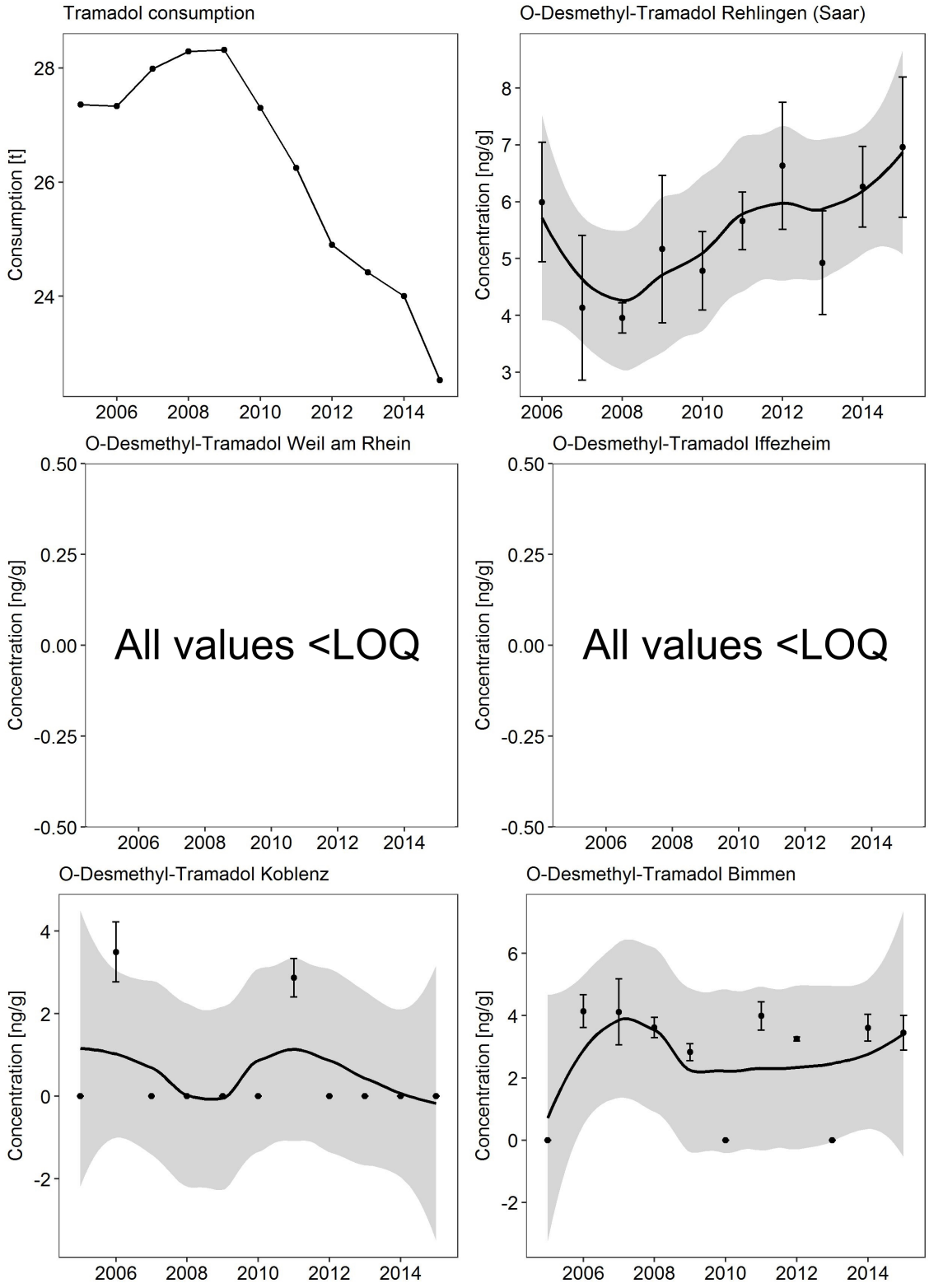

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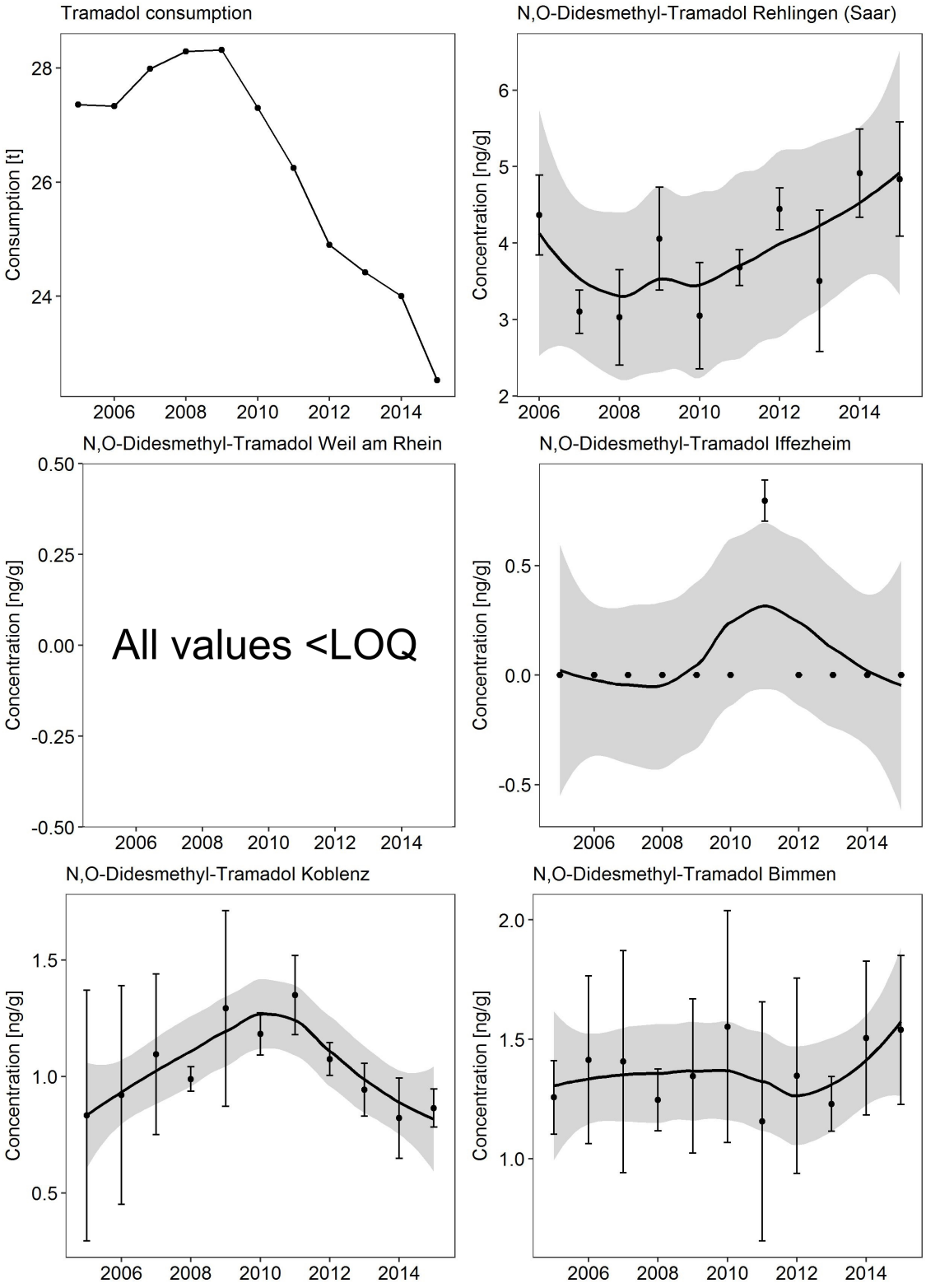

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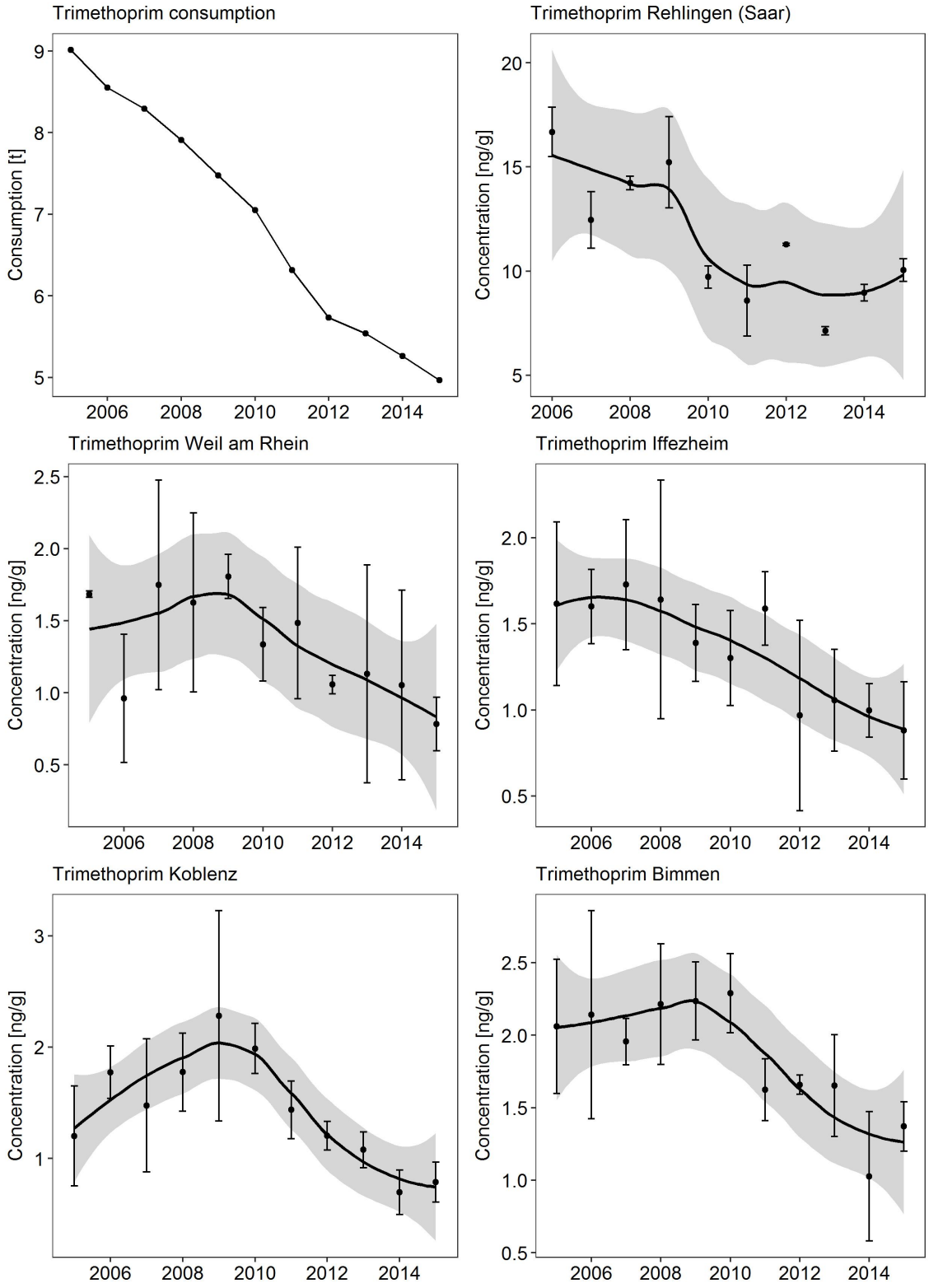


Figure B.5: (continued)

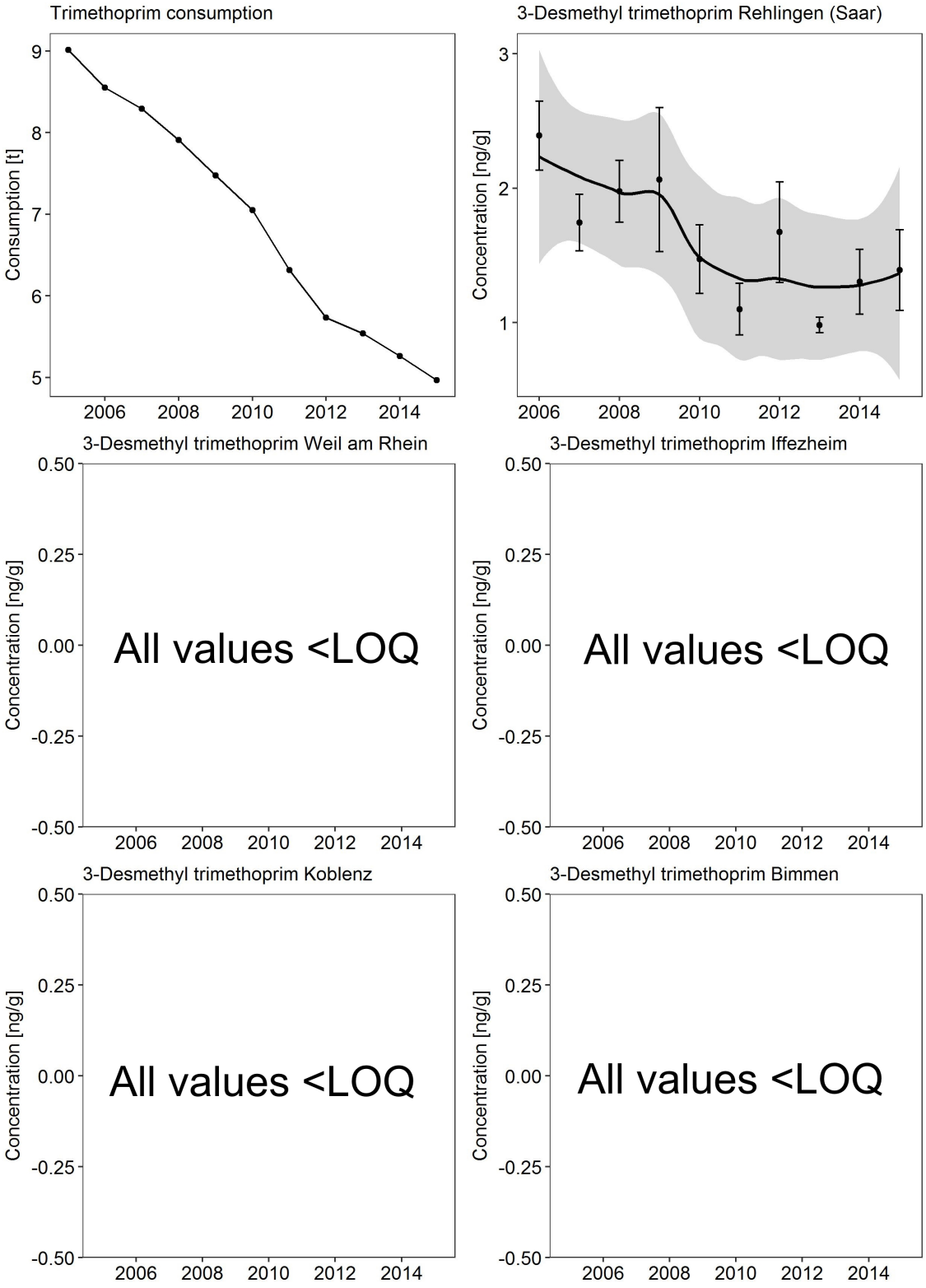


Figure B.5: (continued)

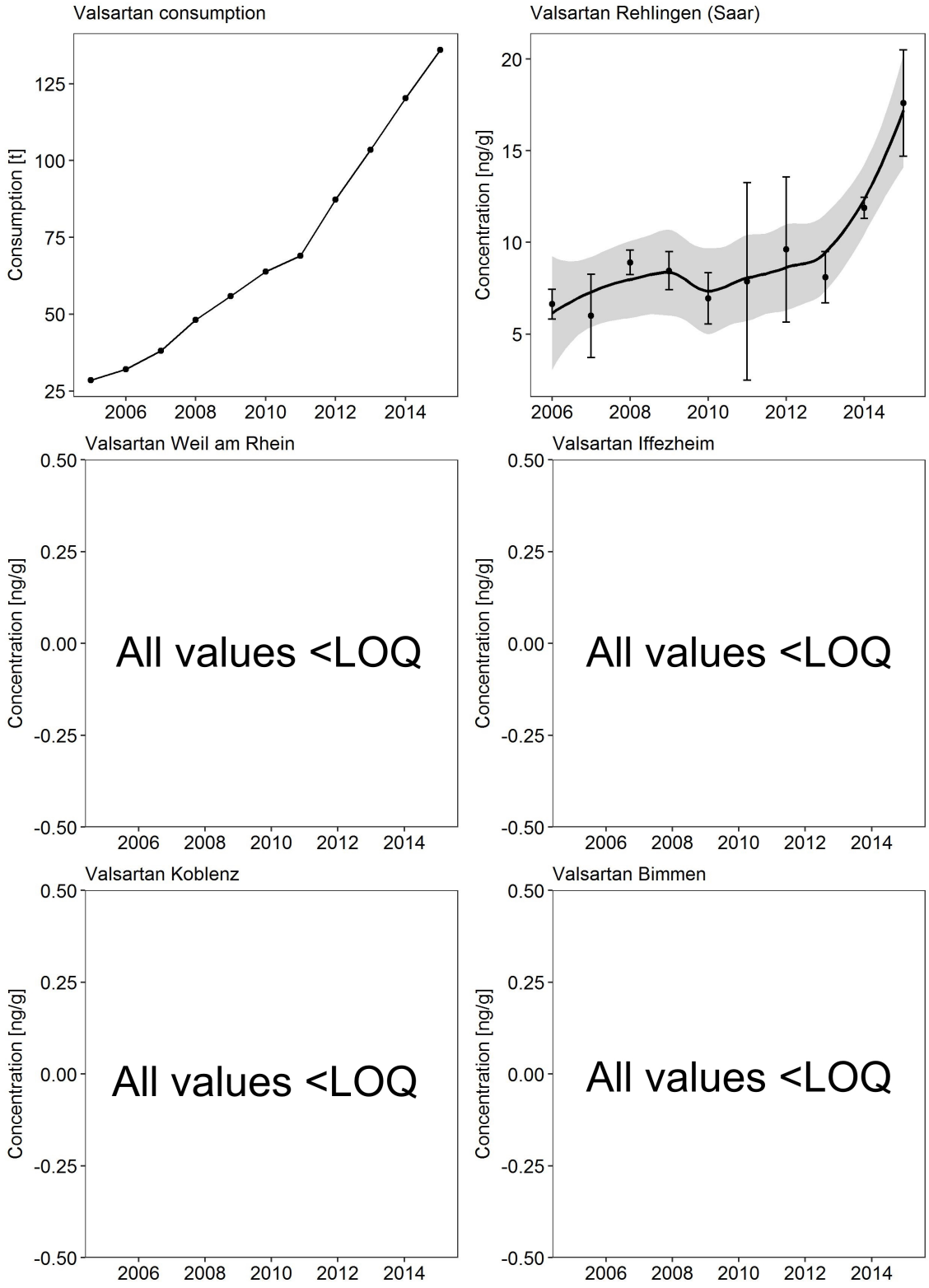


Figure B.5: (continued)

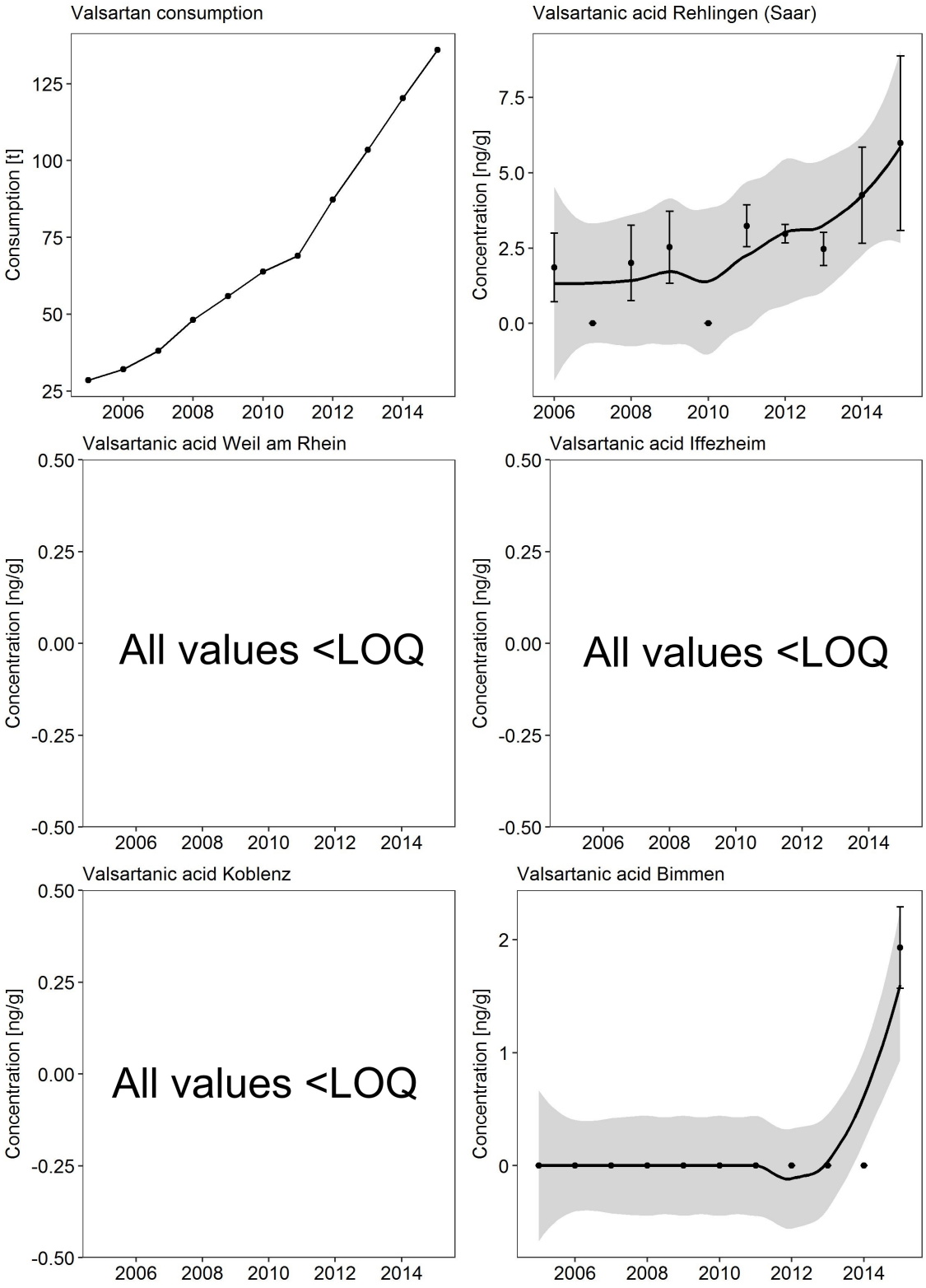
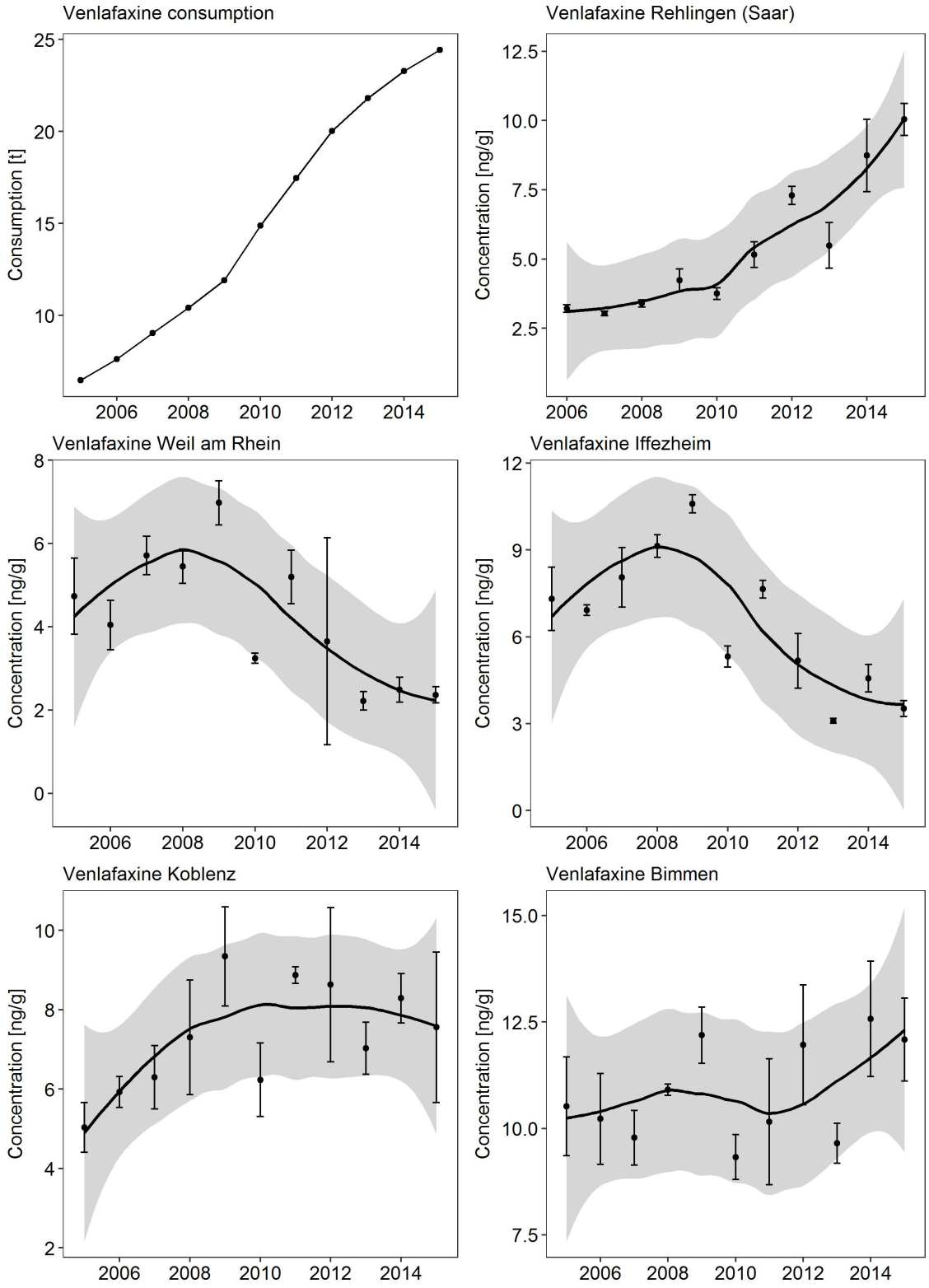


Figure B.5: (continued)



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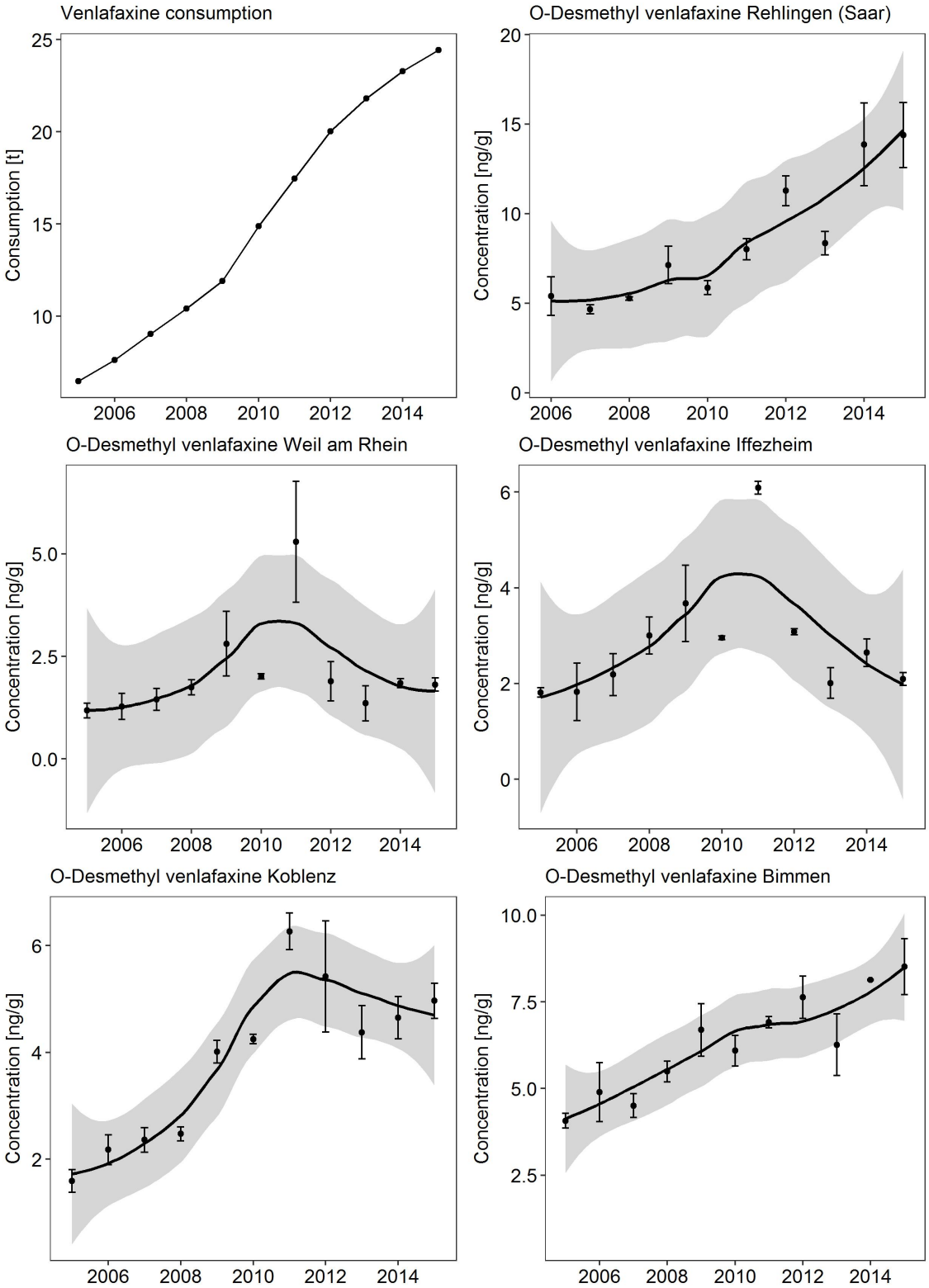


Figure B.5: (continued)

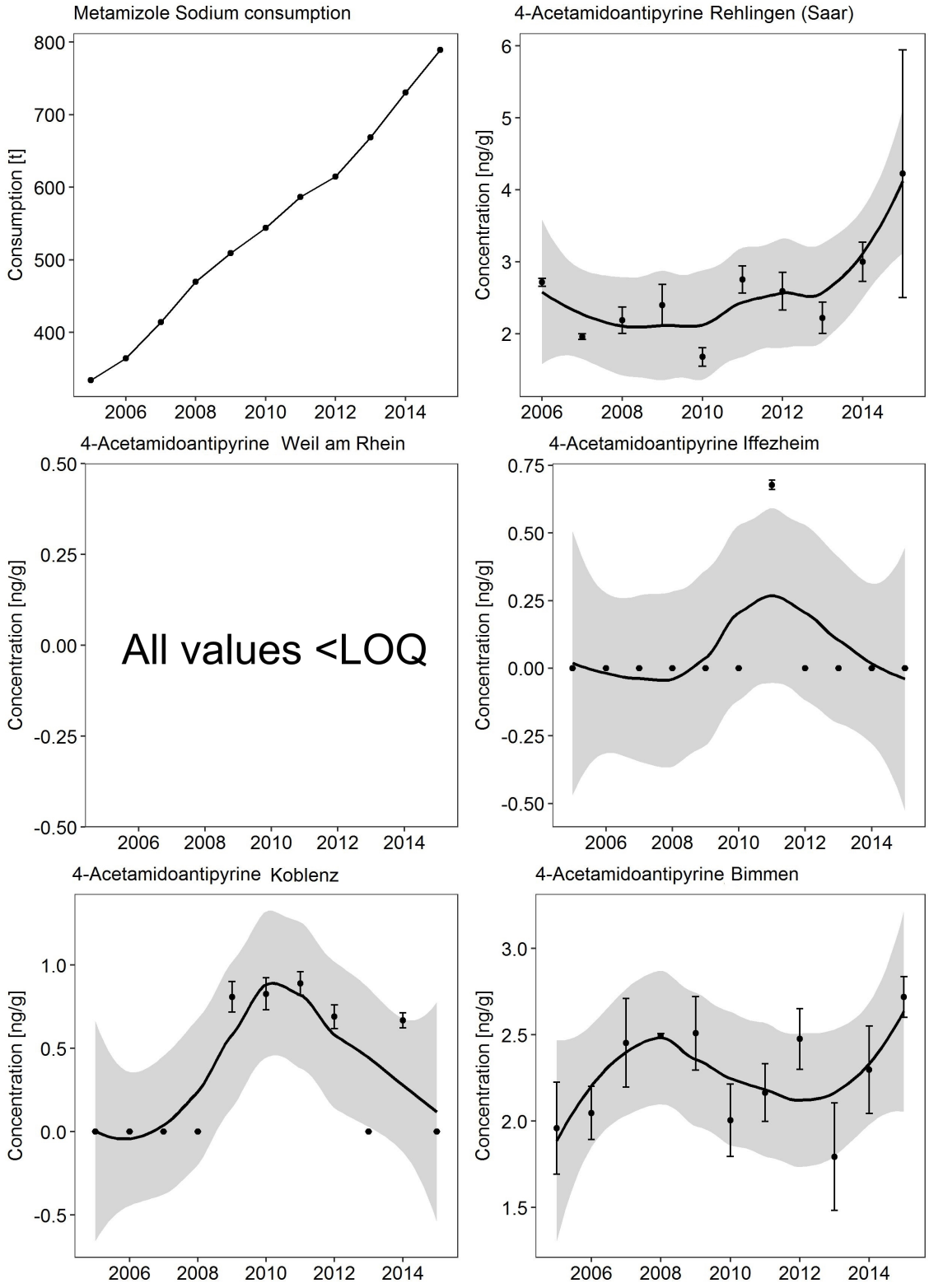
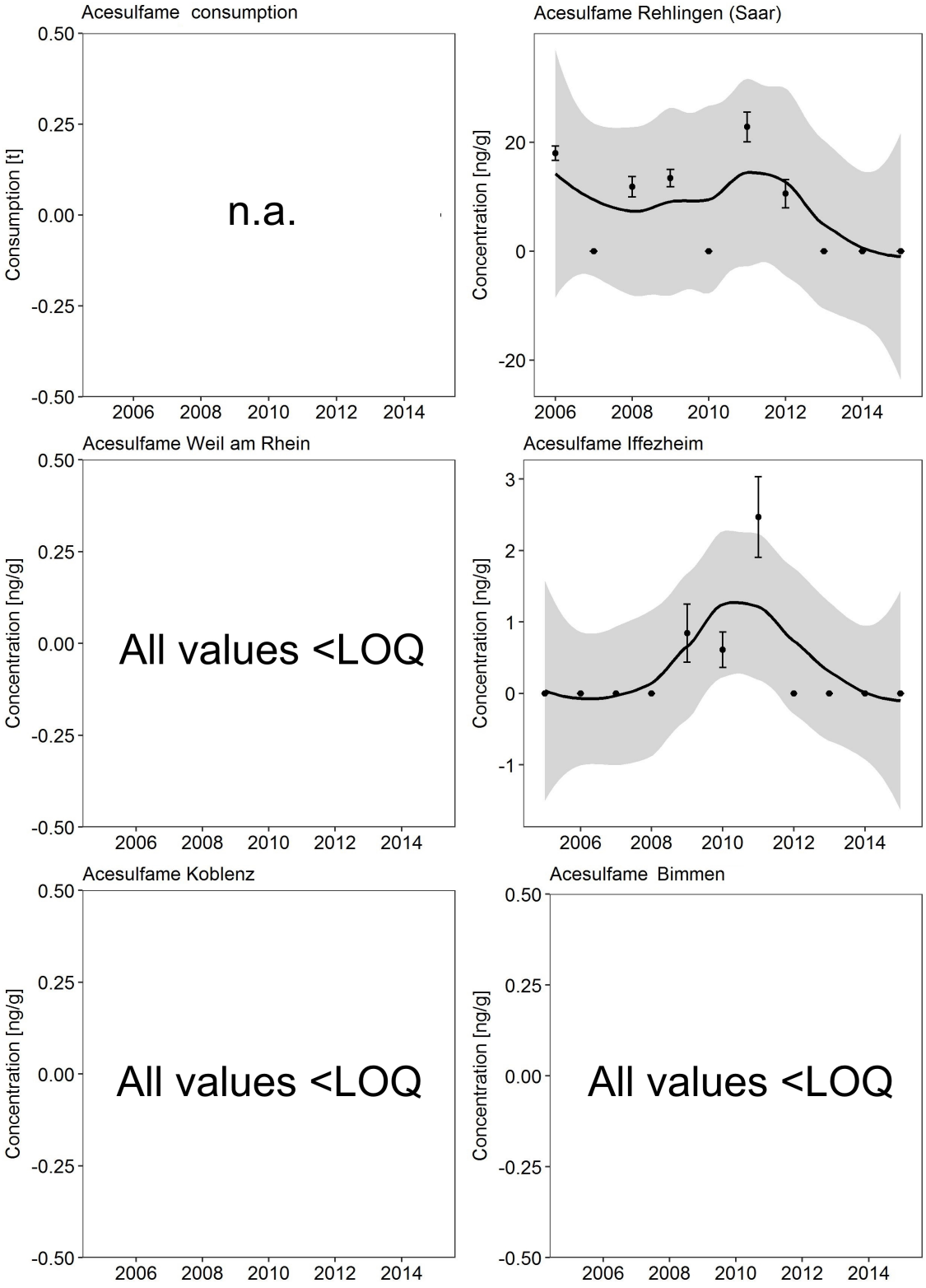


Figure B.5: (continued)



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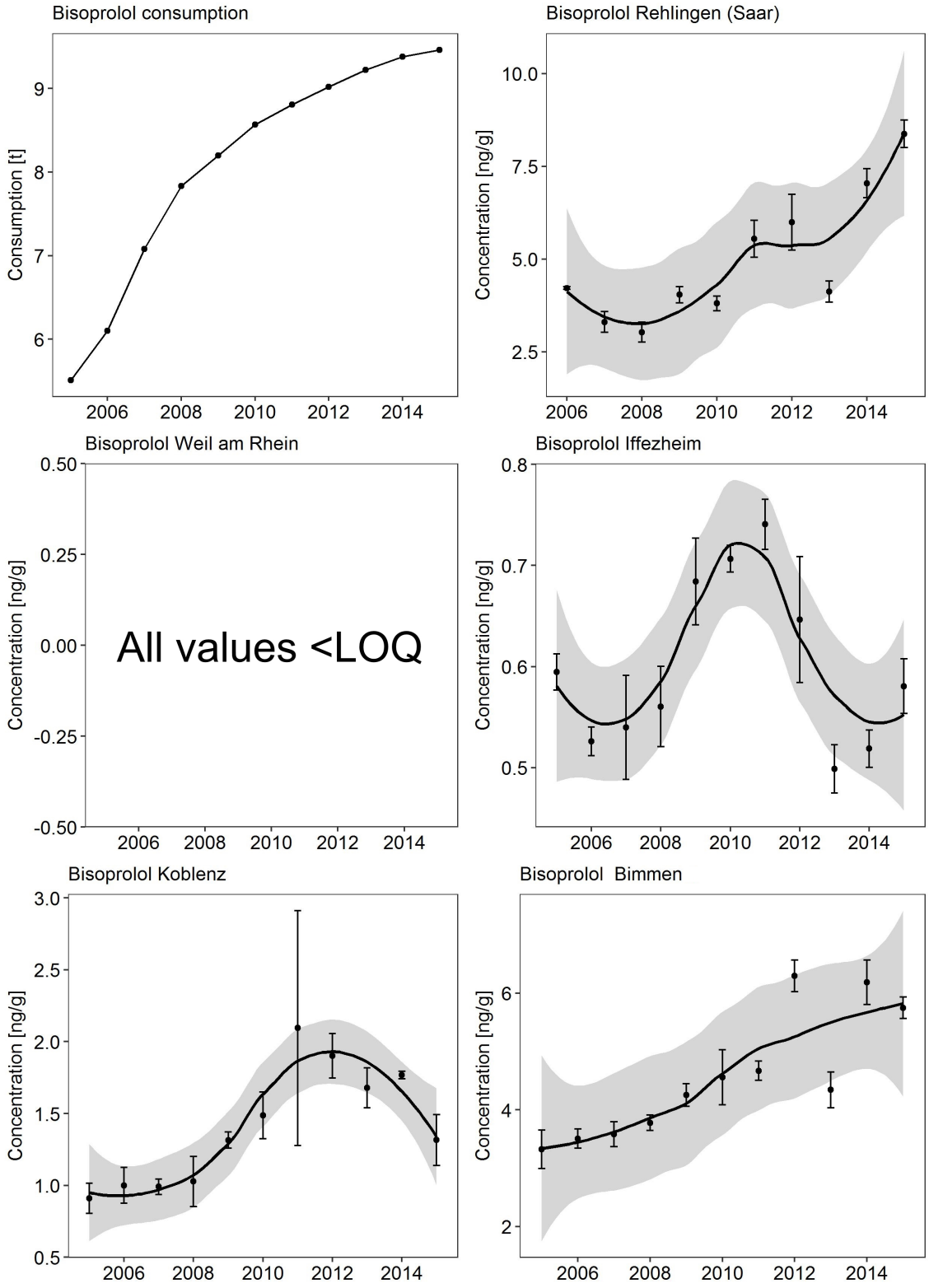

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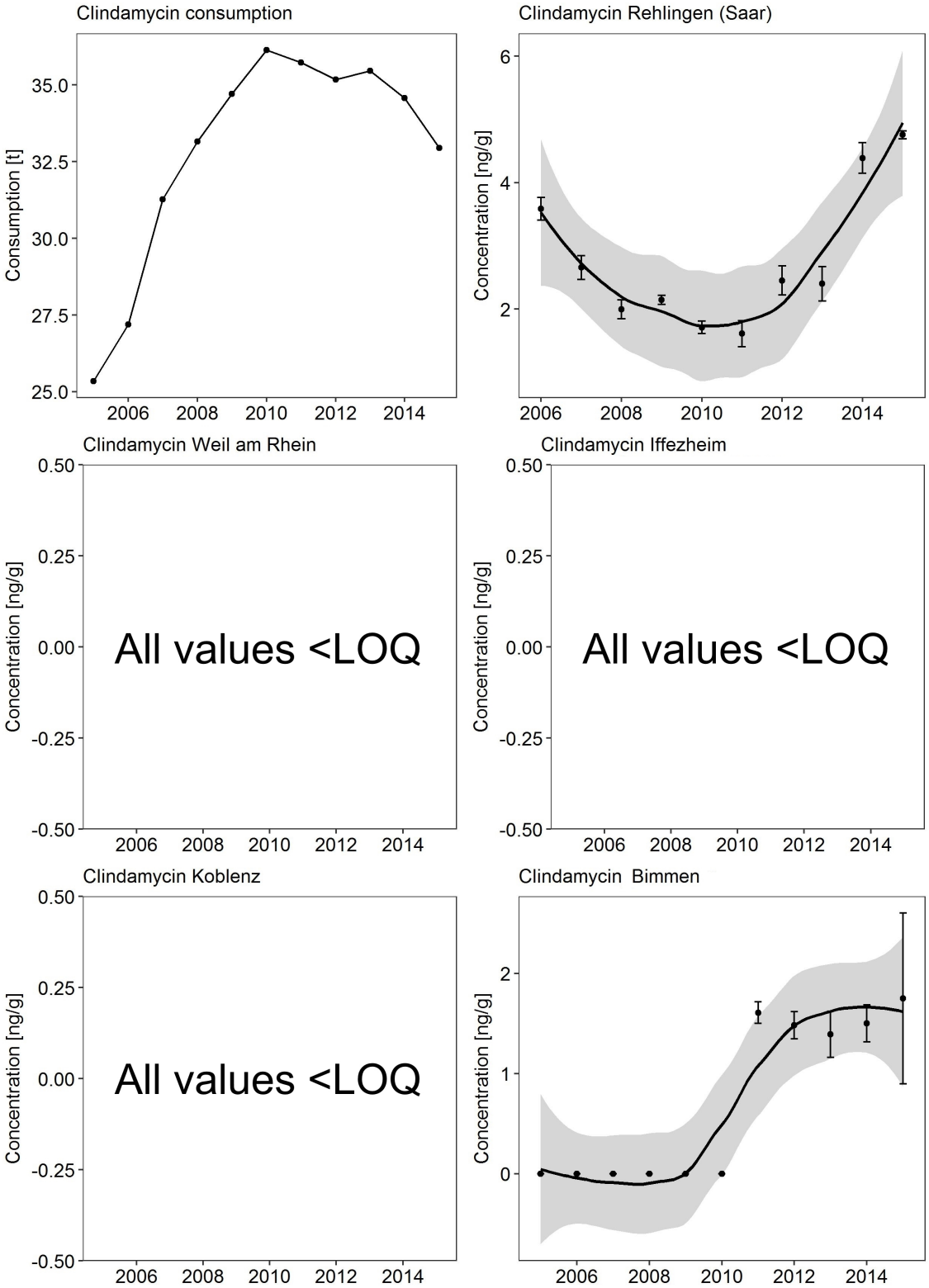

Figure B.5: (continued)

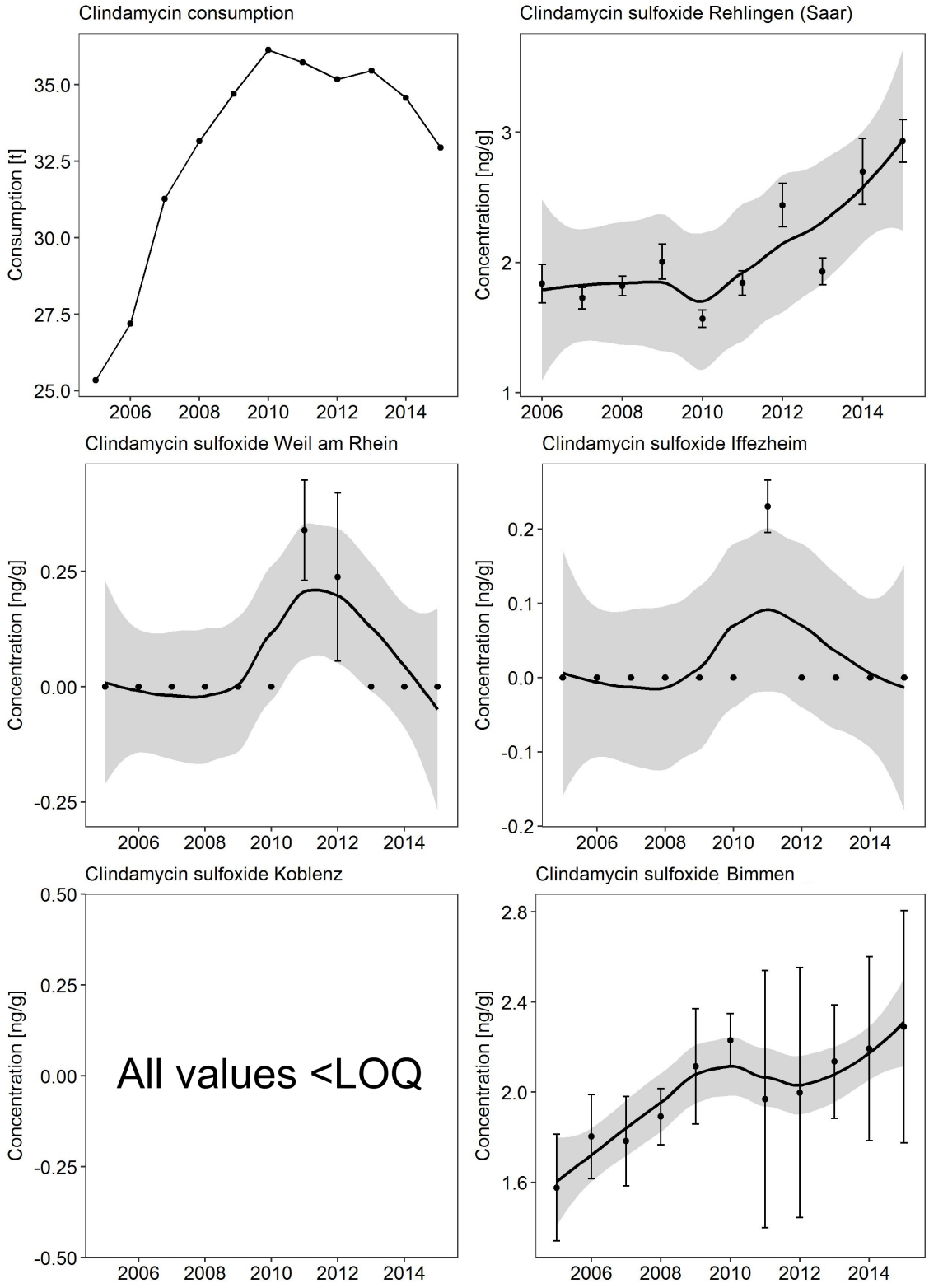

Figure B.5: (continued)

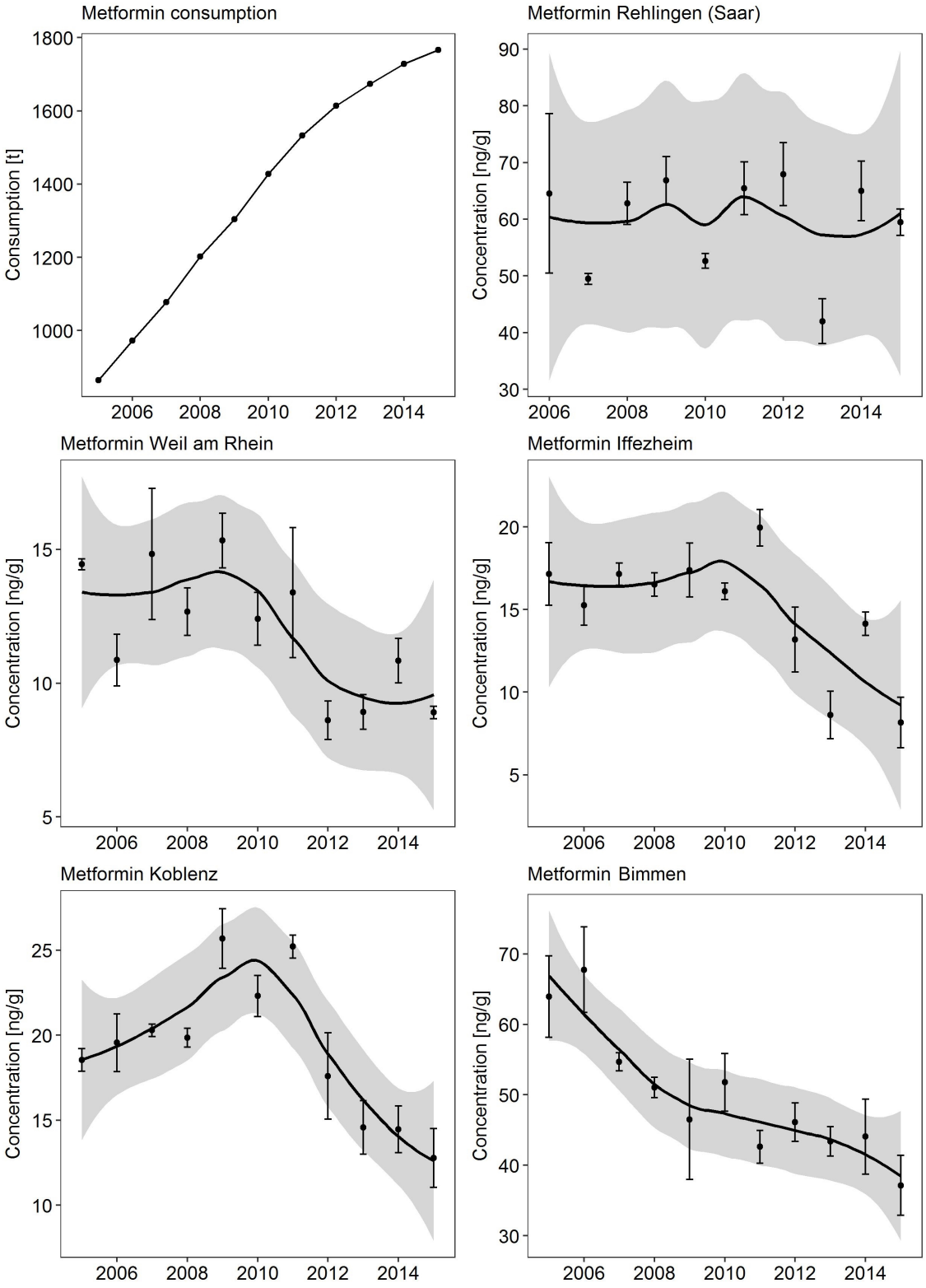

Figure B.5: (continued)

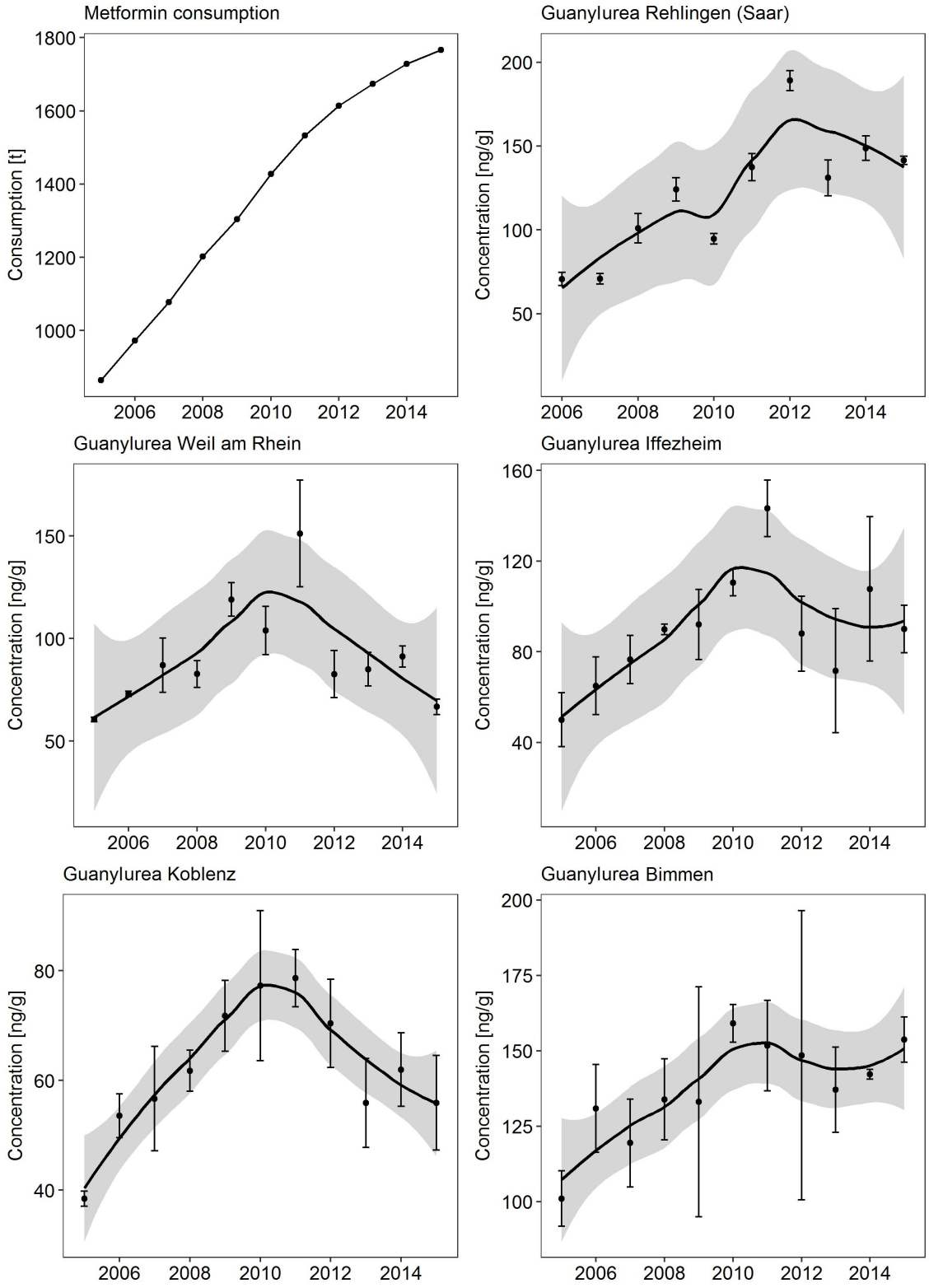

Figure B.5: (continued)

Table B.12: Pearson coefficients and p-values for the correlations between the concentrations in SPM of the metabolites/TP and that of their parent pharmaceutical for the different analytes for the sampling sites Weil am Rhein (Rhine, km 173), Iffezheim (Rhine, km 333), Koblenz (Rhine, km 590), Bimmen (Rhine, km 863) and Rehlingen (Saar, km 54). Pearson coefficients and p-values were only determined when the concentrations were above LOQ for each investigated year.

Analytes	Weil am Rhein (n=11)		Iffezheim (n=11)		Koblenz (n=11)		Bimmen (n=11)		Rehlingen (n=10)	
	Pearson	p-value	Pearson	p-value	Pearson	p-value	Pearson	p-value	Pearson	p-value
Atenolol	-	-	-	-	-	-	-	-	-	-
Atenolol acid	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.90	4.11×10^{-4}
Carbamazepine	-	-	-	-	-	-	-	-	-	-
10,11-dihydroxy-10,11-dihydrocarbamazepine	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.66	0.038
Acridone	0.34	0.31	0.75	0.0078	0.83	0.0016	0.33	0.32	0.33	0.35
9-Acridine carboxylic acid	n.a.	n.a.	n.a.	n.a.	0.32	0.34	0.15	0.65	-0.50	0.14
Citalopram	-	-	-	-	-	-	-	-	-	-
Desmethylcitalopram	0.98	8.7×10^{-8}	0.95	6.2×10^{-6}	0.99	4.7×10^{-9}	1.00	1.6×10^{-10}	1.00	2.1×10^{-9}
Diclofenac	-	-	-	-	-	-	-	-	-	-
4'-Hydroxy-diclofenac	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.63	0.052
Diphenhydramine	-	-	-	-	-	-	-	-	-	-
N-Desmethyl diphenhydramine	n.a.	n.a.	0.86	6.0×10^{-4}	0.78	0.0047	0.76	0.0068	0.98	1.5×10^{-6}
Flecainide	-	-	-	-	-	-	-	-	-	-
Flecainide-meta-O-dealkylated	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.95	2.5×10^{-5}
Fluoxetine	-	-	-	-	-	-	-	-	-	-
Norfluoxetine	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.72	0.020
Metoprolol	-	-	-	-	-	-	-	-	-	-
Hydroxy metoprolol	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.22	0.54
Tramadol	-	-	-	-	-	-	-	-	-	-
O-Desmethyl-Tramadol	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.89	4.8×10^{-4}
N,O-Didesmethyl-Tramadol	n.a.	n.a.	n.a.	n.a.	0.91	9.7×10^{-5}	0.32	0.33	0.84	0.0026
Trimethoprim	-	-	-	-	-	-	-	-	-	-
3-Desmethyl trimethoprim	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.99	7.8×10^{-8}
Valsartan	-	-	-	-	-	-	-	-	-	-
Valsartanic acid	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.89	6.46×10^{-4}

Analytes	Weil am Rhein (n=11)		Iffezheim (n=11)		Koblenz (n=11)		Bimmen (n=11)		Rehlingen (n=10)	
	Pearson	p-value	Pearson	p-value	Pearson	p-value	Pearson	p-value	Pearson	p-value
Venlafaxine	-	-	-	-	-	-	-	-	-	-
O-Desmethyl venlafaxine	0.33	0.32	0.35	0.29	0.76	0.0067	0.67	0.025	0.99	$5.9x10^{-9}$
Clindamycin	-	-	-	-	-	-	-	-	-	-
Clindamycin sulfoxide	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.79	0.0065
Metformin	-	-	-	-	-	-	-	-	-	-
Guanylurea	0.37	0.27	0.30	0.37	0.54	0.085	-0.67	0.024	0.36	0.31

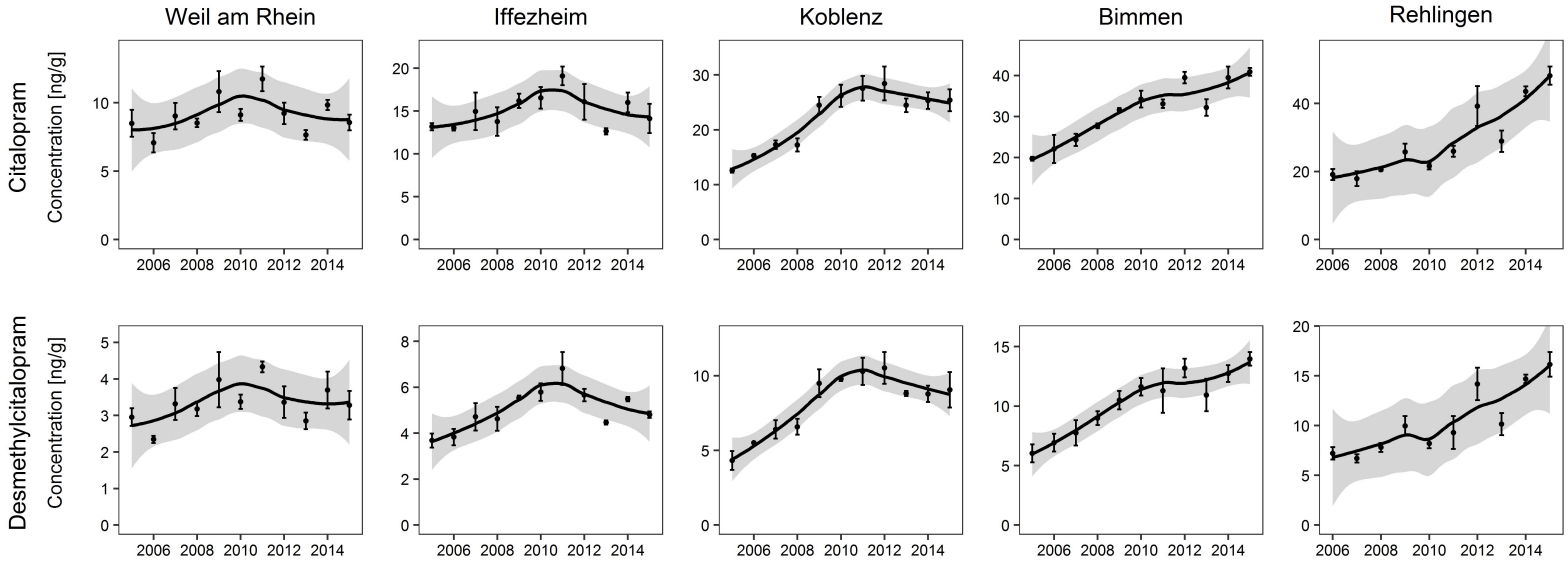

Figure B.6: Temporal concentration trends of citalopram and desmethylcitalopram in SPM from the sampling sites Weil am Rhein (Rhine, km 173), Iffezheim (Rhine, km 333), Koblenz (Rhine, km 590), Bimmen (Rhine, km 863) and Rehlingen (Saar, km 54). The grey shaded area corresponds to the pointwise 95% confidence interval of the LOESS function. Measurements were performed in triplicate and the error bars correspond to the 95% confidence interval.

Table B.13: Pearson coefficients and p-values for the correlation between the consumption quantities and the measured concentrations in SPM for the analytes at from the sampling sites Weil am Rhein (Rhine, km 173), Iffezheim (Rhine, km 333), Koblenz (Rhine, km 590), Bimmen (Rhine, km 863), Rehlingen (Saar, km 54). Pearson coefficients and p-values were only determined when the concentrations were above LOQ for each investigated year

Analytes	Weil am Rhein		Iffezheim		Koblenz		Bimmen		Rehlingen	
	Pearson	p-value	Pearson	p-value	Pearson	p-value	Pearson	p-value	Pearson	p-value
RPLC										
Aliskiren	0.90	$1.6x10^{-4}$	0.92	$6.0x10^{-5}$	0.98	$1.3x10^{-7}$	0.96	$2.8x10^{-6}$	0.73	0.017
Amisulpride	0.59	0.06	0.61	0.046	0.53	0.094	0.51	0.11	-0.01	0.98
Atenolol	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.74	0.014
Atenolol acid	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.69	0.027
Bicalutamide	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.86	$6.8x10^{-4}$	0.75	0.012
Carbamazepine	0.79	0.0040	0.95	$7.6x10^{-6}$	0.93	$3.4x10^{-5}$	0.84	0.0012	0.72	0.019
10.11-dihydroxy-10.11-dihydrocarbamazepine	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.38	0.28
Acridone	0.33	0.30	0.62	0.042	0.66	0.027	0.21	0.54	0.53	0.12
9-Acridine carboxylic acid	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.04	0.91	-0.32	0.37
Cetirizine	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.76	0.0066	0.61	0.061
Citalopram	0.43	0.19	0.53	0.094	0.95	$7.6x10^{-6}$	0.90	$1.6x10^{-4}$	0.67	0.034
Desmethylcitalopram	0.54	0.09	0.74	0.0092	0.92	$6.0x10^{-5}$	0.92	$6.0x10^{-5}$	0.67	0.034
Clarithromycin	0.42	0.20	0.55	0.080	0.59	0.056	0.61	0.046	-0.16	0.66
Clopidogrel	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	-0.07	0.84	0.41	0.24
Diclofenac	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	-0.30	0.40
4'-Hydroxy-diclofenac	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.27	0.45
Diphenhydramine	-0.26	0.40	-0.030	0.93	-0.37	0.26	0.37	0.26	-0.090	0.80
N-Desmethyl diphenhydramine	n.a.	n.a.	0.35	0.29	0.17	0.62	0.83	0.0016	-0.13	0.72
Fexofenadine	n.a.	n.a.	n.a.	n.a.	-0.21	0.54	-0.21	0.54	0.36	0.31
Flecainide	0.73	0.011	0.55	0.08	0.90	$1.6x10^{-4}$	0.93	$3.4x10^{-5}$	0.71	0.021
Flecainide-meta-O-dealkylated	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.72	0.019
Fluoxetine	0.32	0.30	0.41	0.21	0.51	0.11	0.53	0.094	0.52	0.12
Gabapentin lactam	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.82	0.0037
Hydrochlorothiazide	0.35	0.29	-0.27	0.42	0.63	0.038	0.48	0.14	-0.45	0.19

Analytes	Weil am Rhein		Iffezheim		Koblenz		Bimmen		Rehlingen	
	Pearson	p-value	Pearson	p-value	Pearson	p-value	Pearson	p-value	Pearson	p-value
Irbesartan	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.020	0.96
Lidocaine	n.a.	n.a.	-0.42	0.20	0.70	0.016	0.72	0.012	0.59	0.073
Metoprolol	n.a.	n.a.	-0.23	0.50	0.88	$3.5x10^{-4}$	0.72	0.012	0.30	0.40
Hydroxy metoprolol	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	-0.69	0.027
Roxithromycin	n.a.	n.a.	n.a.	n.a.	0.51	0.11	0.89	$2.4x10^{-4}$	0.24	0.50
Sertraline	n.a.	n.a.	0.88	$3.5x10^{-4}$	n.a.	n.a.	0.88	$3.5x10^{-4}$	0.65	0.042
Sitagliptin	1.00	$4x10^{-13}$	0.99	$5.8x10^{-9}$	0.99	$5.8x10^{-9}$	1.00	$2.5x10^{-12}$	0.98	$6.8x10^{-7}$
Sotalol	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.81	0.0045
Sulpiride	0.80	0.0031	0.88	$3.5x10^{-4}$	0.64	0.034	0.94	$1.7x10^{-5}$	0.64	0.046
Telmisartan	0.81	0.0025	0.94	$1.7x10^{-5}$	0.79	0.0038	0.81	0.0025	0.59	0.073
Torasemide	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.74	0.014
Tramadol	0.88	$3.8x10^{-4}$	0.89	$2.7x10^{-4}$	0.37	0.27	0.25	0.47	-0.73	0.011
O-Desmethyl-Tramadol	n.a.	n.a.	n.a.	n.a.	0.27	0.42	0.56	0.073	-0.60	0.067
N.O-Didesmethyl-Tramadol	n.a.	n.a.	n.a.	n.a.	0.36	0.28	0.17	0.62	-0.45	0.19
Trimethoprim	0.65	0.03	0.89	$2.4x10^{-4}$	0.60	0.051	0.80	0.0031	0.79	0.0065
3-Desmethyl trimethoprim	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.77	0.0092
Valsartan	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.83	0.0030
Venlafaxine	-0.70	0.016	-0.74	0.0092	0.54	0.086	0.41	0.21	0.90	$3.6x10^{-4}$
O-Desmethyl venlafaxine	0.19	0.60	0.14	0.67	0.82	0.002	0.90	$1.3x10^{-4}$	0.89	$5.6x10^{-4}$
HILIC										
4-Acetylaminoantipyrine	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.29	0.39	0.63	0.051
Bisoprolol	n.a.	n.a.	0.17	0.62	0.77	0.0056	0.81	0.0025	0.63	0.051
Clindamycin	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	-0.42	0.23
Clindamycin sulfoxide	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.78	0.0046	0.08	0.83
Metformin	-0.65	0.03	-0.58	0.061	-0.42	0.20	-0.91	$1.0x10^{-4}$	-0.040	0.91
Guanylurea	0.25	0.50	0.56	0.073	0.47	0.14	0.78	0.0046	0.82	0.0037

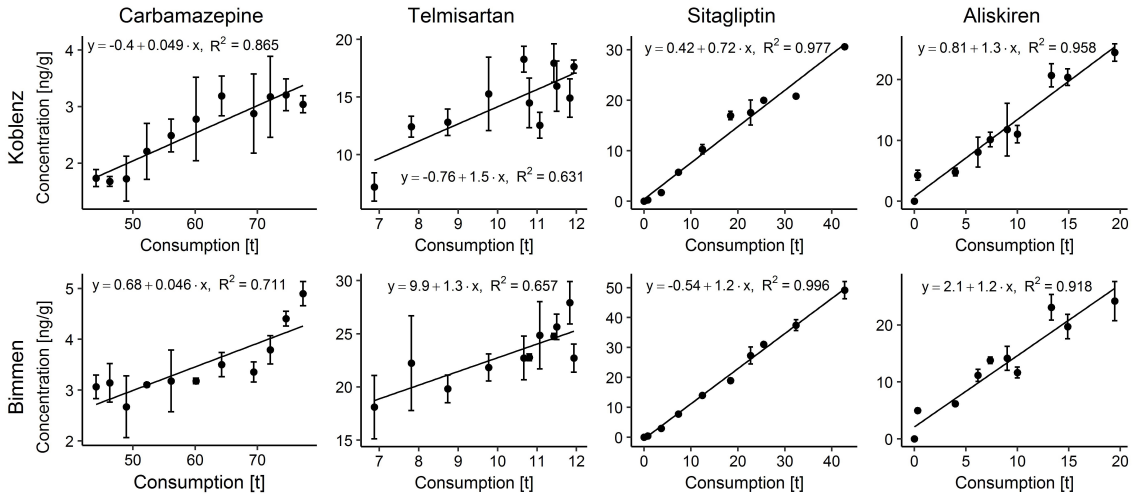

Figure B.7: Relations between the concentration of carbamazepine, telmisartan, sitagliptin and aliskiren in SPM and their respective consumptions in Germany between 2005 and 2015. Errors bar correspond to the 95% confidence interval

Table B.14: Parameters of the linear relationship between the consumption quantities and the measured concentrations in SPM for the analytes at from the sampling sites Weil am Rhein (Rhine, km 173), Iffezheim (Rhine, km 333), Koblenz (Rhine, km 590), Bimmen (Rhine, km 863), Rehlingen (Saar, km 54), for the analytes with Pearson coefficients r>0.6

	Weil am Rhein			Iffezheim			Koblenz			Bimmen			Rehlingen		
Name	Slope	Intercept	R^2	Slope	Intercept	R^2	Slope	Intercept	R^2	Slope	Intercept	R^2	Slope	Intercept	R^2
RPLC															
Aliskiren	0.61	1.22	0.81	1.24	1.62	0.85	1.26	0.81	0.96	1.25	2.12	0.92	1.78	4.23	0.54
Amisulpride	r<0.6	r<0.6	r<0.6	0.97	0.04	0.37	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6
Atenolol	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.16	0.46	0.55
Atenolol acid	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.38	1.58	0.48
Bicalutamide	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.3	0.25	0.74	1.00	0.02	0.57
Carbamazepine	0.03	-0.52	0.62	0.07	-2.06	0.9	0.05	-0.4	0.87	0.05	0.68	0.71	0.11	0.06	0.52
10,11-Dihydroxy-10.11-dihydrocarbamazepine	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	r<0.6	r<0.6	r<0.6
Acridone	r<0.6	r<0.6	r<0.6	0.02	1.71	0.38	0.02	2.11	0.44	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6
9-Acridine carboxylic acid	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6
Cetirizine	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.55	2.24	0.58	1.82	3.24	0.38
Citalopram	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	2.3	5.51	0.9	2.85	10.61	0.81	3.56	1.89	0.44
Desmethylcitalopram	r<0.6	r<0.6	r<0.6	0.3	2.86	0.54	0.84	2.03	0.84	1.06	2.68	0.84	1.12	1.87	0.45
Clarithromycin	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	0.94	-1.43	0.38	r<0.6	r<0.6	r<0.6
Clopidogrel	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6
Diclofenac	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	r<0.6	r<0.6	r<0.6
4'-Hydroxy-diclofenac	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	r<0.6	r<0.6	r<0.6
Diphenhydramine	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6
N-Desmethyl diphenhydramine	n.a.	n.a.	n.a.	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	2.19	-0.89	0.69	r<0.6	r<0.6	r<0.6
Fexofenadine	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6
Flecainide	0.18	0.12	0.54	0.5	1.14	0.3	1.18	-0.44	0.81	2.25	-0.11	0.86	7.48	-8.64	0.51
Flecainide-meta-O-dealkylated	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.19	-0.04	0.51
Fluoxetine	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6
Gabapentin lactam	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.04	0.26	0.67
Hydrochlorothiazide	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	0.1	-2.91	0.4	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6

	Weil am Rhein			Iffezheim			Koblenz			Bimmen			Rehlingen		
Name	Slope	Intercept	R^2	Slope	Intercept	R^2	Slope	Intercept	R^2	Slope	Intercept	R^2	Slope	Intercept	R^2
Irbesartan	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	r<0.6	r<0.6	r<0.6
Lidocaine	n.a.	n.a.	n.a.	r<0.6	r<0.6	r<0.6	0.07	-0.09	0.49	0.11	-0.26	0.51	r<0.6	r<0.6	r<0.6
Metoprolol	n.a.	n.a.	n.a.	r<0.6	r<0.6	r<0.6	0.13	-14.88	0.77	0.12	-11.44	0.52	r<0.6	r<0.6	r<0.6
Hydroxy metoprolol	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	r<0.6	r<0.6	r<0.6
Roxithromycin	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	r<0.6	r<0.6	r<0.6	0.88	-0.9	0.79	r<0.6	r<0.6	r<0.6
Sertraline	n.a.	n.a.	n.a.	0.21	1.29	0.77	n.a.	n.a.	n.a.	0.49	0.69	0.78	0.85	2.37	0.42
Sitagliptin	0.36	-0.05	1.00	0.36	0.06	0.98	0.72	0.42	0.98	1.17	-0.54	1.00	2.21	-3.69	0.97
Sotalol	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.32	2.73	0.66
Sulpiride	0.14	0.06	0.64	0.24	0.19	0.77	0.57	1.13	0.41	0.76	1.45	0.88	1.95	3.44	0.41
Telmisartan	0.7	-3.67	0.66	0.97	-4.68	0.88	1.49	-0.76	0.63	1.29	9.87	0.66	8.99	-21.76	0.34
Torasemide	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.03	-0.07	0.54
Tramadol	-10.92	0.52	0.77	-16.99	0.85	0.79	-27.23	1.14	0.74	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6
O-Desmethyl tramadol	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6
N,O-Didesmethyl tramadol	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6
Trimethoprim	0.16	0.22	0.42	0.2	-0.01	0.78	0.21	-0.01	0.36	0.23	0.27	0.64	1.87	-1.11	0.62
3-Desmethyl trimethoprim	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.26	-0.14	0.6
Valsartan	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.08	3.19	0.69
Venlafaxine	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	0.36	-0.36	0.81
O-Desmethyl venlafaxine	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.52	0.10	0.80
HILIC															
4-Acetylaminoantipyrine	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	r<0.6	r<0.6	r<0.6	0.003	0.74	0.39
Bisoprolol	n.a.	n.a.	n.a.	r<0.6	r<0.6	r<0.6	0.23	-0.49	0.59	0.64	-0.62	0.65	1.01	-3.51	0.40
Clindamycin	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	r<0.6	r<0.6	r<0.6
Clindamycin sulfoxide	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.05	0.42	0.62	r<0.6	r<0.6	r<0.6
Metformin	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6
Guanylurea	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	r<0.6	0.04	80.03	0.61	0.11	-34.13	0.67

C

Supplementary data for chapter 4: Development of an analytical method to quantify pharmaceuticals in fish tissues by LC-MS/MS detection

Table C.1: List of selected analytes, application and log D at pH 7

Name	Cas No	Application	Charge at pH 7[a]	Log D at pH 7[a]
Amisulpride	71675-85-9	Antipsychotics	Majoritary positive (minoritary form: neutral)	-0.076
Bezafibrate	41859-67-0	Lipid regulating agent	Negative	0.973
Bicalutamide	90357-06-5	Antiandrogen	Neutral	2.709
Candesartan	139481-59-7	Angiotensin	Negative	1.039
Carbamazepine	298-46-4	Antiepileptic	Neutral	2.766
2-Hydroxycarbamazepine	68011-66-5	Metabolite of carbamazepine [327]	Negative	1.370
3-Hydroxycarbamazepine	68011-67-6	Metabolite of carbamazepine [327]	Negative	1.353
10,11-Dihydro-10-hydroxy-carbamazepine	29331-92-8	Metabolite of carbamazepine [277]	Neutral	1.732
10,11-Dihydroxy-10,11-dihydrocarbamazepine	29331-92-8	Main human metabolite of carbamazepine [217]	Neutral	0.813
Acridone	578-95-0	Metabolite of carbamazepine, TP of 10,11-dihydro-10-hydroxy-carbamazepine and 10,11-dihydroxy-10,11-dihydrocarbamazepine [277]	Neutral	4.199
Cetirizine	83881-51-0	Antihistamine	Majoritary zwitterion (minoritary form: negative)	0.772
Chlorothiazide	58-94-6	Diuretic	Neutral	-0.445
Citalopram	59729-33-8	Antidepressant	Positive	1.055
Desmethylcitalopram	62498-67-3	Metabolite of citalopram [328]	Positive	0.317
Didemethylcitalopram	62498-69-5	Metabolite of citalopram [328]	Positive	0.140
Clopidogrel	113665-84-2	Cardiac agent	Neutral	4.028
Clopidogrel carboxylic acid	144457-28-3	Main human metabolite of clopidogrel [329]	Majoritary zwitterion (minoritary form: negative)	1.212
Diclofenac	15307-86-5	Nonsteroidal anti-inflammatory drug	Negative	1.368
Diphenhydramine	58-73-1	Antihistamine	Positive	1.790
N-Desmethyl diphenhydramine	53499-40-4	Main human metabolite [331] and anareobic TP [133]	Positive	0.691
Fexofenadine	83799-24-0	Antihistamine	Zwitterion	2.940
Flecainide	54143-55-4	Antiarrhythmic agent	Positive	0.663
Flecainide-meta-O-dealkylated	83526-33-4	Main metabolite of flecainide [60]	Positive	-0.391
Fluconazole	86386-73-4	Antifungal	Neutral	0.561
Fluoxetine	54910-89-3	Antidepressant	Positive	1.504
Norfluoxetine	56161-73-0	Main metabolite of fluoxetine [62]	Positive	1.160
Furosemide	54-31-9	Cardiac agent/diuretic	Negative	-0.930

Name	Cas No	Application	Charge at pH 7[a]	Log D at pH 7[a]
Gabapentin lactam	64744-50-9	TP of gabapentin [322]	Neutral	1.033
Hydrochlorothiazide	58-93-5	Diuretic agent	Neutral	-0.579
Lamotrigine	84057-84-1	Antiepileptic	Majoritary neutral (minoritary form: positive)	1.894
Lidocaine	137-58-6	Local anesthetic	Majoritary positive (minoritary form: neutral)	2.019
Norlidocaine	7729-94-4	Metabolite of lidocaine [71]	Majoritary positive (minoritary form: neutral)	0.520
Metoprolol	37350-58-6	Beta-blocker	Positive	0.807
O-Desmethyl metoprolol	62572-94-5	Metabolite of metoprolol and TP of metoprolol [119]	Positive	-1.450
Oxazepam	604-75-1	Benzodiazepine	Neutral	2.923
Phenytoin	57-41-0	Anticonvulsants	Majoritary neutral (minoritary form: negative)	2.134
Pregabalin lactam	148553-50-8	TP of the antiepileptic pregabalin	Zwitterionic	-1.346
Primidone	125-33-7	Anticonvulsants	Neutral	1.118
Quetiapine	111974-69-7	Antipsychotics	Majoritary neutral (minoritary form: positive)	2.474
Quetiapine sulfoxide	329216-63-9	Major human metabolite of quetiapine [332]	Majoritary neutral (minoritary form: positive)	1.222
Sertraline	79617-96-2	Antidepressant	Positive	2.668
Sitagliptin	486460-32-6	Antidiabetic drug	Majoritary positive (minoritary form: neutral)	-0.510
Sulfamethoxazole	723-46-6	Antibiotic	Majoritary negative (minoritary form: neutral)	0.140
N-Acetyl sulfamethoxazole	21312-10-7	Metabolite of sulfamethaxole [334]	Majoritary negative (minoritary form: neutral)	0.104
Sulpiride	23672-07-3	Antipsychotic	Majoritary positive (minoritary form: neutral)	-1.074
Telmisartan	144701-48-4	Angiotensin	Majoritary negative (minoritary form: zwitterion)	5.167
Torasemide	56211-40-6	Diuretic	Majoritary negative (minoritary form: neutral)	1.220
Hydroxytorasemide	99300-68-2	Metabolite of torasemide [89]	Majoritary negative (minoritary form: neutral)	-0.057
Tramadol	36282-47-0	Analgesic	Positive	0.239
O-Desmethyl tramadol	73986-53-5	Metabolite of tramadol [91]	Positive	0.103
N-Desmethyl tramadol	147762-58-1	Metabolite of tramadol [91]	Positive	-0.664
N,O-Didesmethyl tramadol	138853-73-3	Metabolite of tramadol [91]	Positive	-0.743
Trimethoprim	738-70-5	Antibiotic	Majoritary positive (minoritary form: neutral)	0.920
3-Desmethyl trimethoprim	27653-69-6	Metabolite of trimethoprim [93]	Majoritary positive (minoritary form: neutral)	0.772
5-(3,4,5-Trimethoxybenzoyl)-2,4-pyrimidinediamine	30806-86-1	TP of trimethoprim [344]	Majoritary positive (minoritary form: neutral)	1.230
Valsartan	137862-53-4	Angiotensin	Negative	1.646

Name	Cas No	Application	Charge at pH 7[a]	Log D at pH 7[a]
Valeryl-4-hydroxyvalsartan	188259-69-0	Metabolite of valsartan [94]	Negative	0.122
Valsartanic acid	164265-78-5	TP of Valsartan [335]	Negative	-0.727
Venlafaxine	93413-69-5	Antidepressant	Positive	0.836
N-Desmethyl venlafaxine	149289-30-5	Metabolite of venlafaxine [95]	Positive	-0.296
O-Desmethyl venlafaxine	93413-62-8	Metabolite of venlafaxine [95]	Majoritary positive (minoritary form: neutral)	0.687
N,O-Desmethyl venlafaxine	135308-74-6	Metabolite of venlafaxine [95]	Positive	-0.431
Xipamide	14293-44-8	Diuretic	Negative	1.190

[a] https://chemicalize.com/

Table C.2: Concentrations determined in fish tissues by other authors for the investigated analytes. Non-detections are only mentioned if they are the only reported measurement for the substance in the tissue or if the authors detected the concerned analyte in another tissue. We do not included studies concerning market fish. n.d.: not detected, LOQ: limit of quantification, LOD: limit of detection. X: detected

								Detected in		
Analyte	Concentration [ng/g]	Sampling location	Country	Fish species	Analyzed tissue	Quantification limit [ng/g]	Source	Rhine/ Saar fish	Teltow Canal fish	WWTP effluent ponds fish
Amisulpride	-	-	-	-	-	-	-	-	-	X
Bezafibrate	n.d. (w.w.)	WWTP effluent dominated stream	Japan	Crucian carp, common carp	Liver	LOQ: 0.27[a]	[285]	-	-	-
	n.d.-0.14	WWTP effluent dominated stream	Japan	Crucian carp, common carp	Plasma	LOQ: 0.062[a]	[285]			
	n.d. (w.w.)	WWTP effluent dominated stream	Japan	Crucian carp, common carp	Fillet	LOD: 0.033[b]	[305]			
	n.d. (w.w.)	WWTP effluent dominated stream	Japan	Crucian carp, common carp	Liver	LOD: 0.26[b]	[305]			
	n.d-0.139	WWTP effluent dominated stream	Japan	Crucian carp, common carp	Plasma	LOD: 0.0051[b]	[305]			
Bicalutamide	-	-	-	-	-	-	-	-	X	X
Candesartan	-	-	-	-	-	-	-	-	-	-
Carbamazepine	n.d.-0.11 (w.w.)	WWTP effluent dominated stream	Japan	Crucian carp, common carp	Liver	LOQ: 0.18[a]	[285]	-	X	X
	0.030-0.055	WWTP effluent dominated stream	Japan	Crucian carp, common carp	Plasma	LOQ: 0.020[a]	[285]			
	n.d.- 0.0500 (w.w.)	WWTP effluent dominated stream	Japan	Crucian carp, common carp	Fillet	LOD: 0.012[b]	[305]			
	n.d-0.204 (w.w.)	WWTP effluent dominated stream	Japan	Crucian carp, common carp	Liver	LOD:0.048[b]	[305]			
	n.d.-0.0545	WWTP effluent dominated stream	Japan	Crucian carp, common carp	Plasma	LOD: 0.010[b]	[305]			
	n.d.-0.60 (w.w.)	Downstream WWTP	USA	Brown trout	Fillet	LOD: 0.55[b]	[307]			

Analyte	Concentration [ng/g]	Sampling location	Country	Fish species	Analyzed tissue	Quantification limit [ng/g]	Source	Detected in: Rhine/ Saar fish	Detected in: Teltow Canal fish	Detected in: WWTP effluent ponds fish
	n.d.-1.1 (w.w.)	Downstream WWTP	USA	Brown trout	Liver	LOD: 0.94[b]	[307]			
	n.d. (w.w.)	WWTP effluent dominated pond	Czech republic	Common carp	Fillet	LOQ: 0.22-0.38[c]	[287]			
	0.95 ± 0.29 (w.w.)	WWTP effluent dominated pond	Czech republic	Common carp	Liver	LOQ: 0.24-0.33[c]	[287]			
	0.35 ± 0.11	WWTP effluent dominated pond	Czech republic	Common carp	Plasma	LOQ: 0.18-0.36[c]	[287]			
	n.d.-8.2 (d.w.)	Polluted river sites	USA	Smallmouth bass, largemouth bass, yellow perch, white sucker	Fillet	LOQ: 0.1[a]	[301]			
	0.83-1.44 (w.w.)	Effluent dominated stream	USA	*Lepomis*	Fillet	LOD: 0.54[b]	[309]			
	n.d.-0.88 (w.w.)	River	Uruguay	Streaked prochilos, boga, dorado	Fillet	LOQ: 0.092-0.18	[303]			
	n.d.-6.3 (d.w.)	Lagoon	Spain	Golden grey mullet	Fillet	LOQ: 0.3[a]	[302]			
	n.d.-0.4 (d.w.)	Lagoon	Spain	Black goby	Fillet	LOQ: 0.3[a]	[302]			
	n.d.-2.6 (d.w.)	Lagoon	Spain	Golden grey mullet	Liver	LOQ: 0.3[a]	[302]			
	17.9 ± 2.4 (d.w.)	River	Spain	Salmo trutta	Liver	LOQ: 0.25[a]	[284]			
	n.d.-0.66 (w.w.)	Freshwater pond	USA	Mosquito fish	Whole body	LOD: 0.1[b]	[345]			
	0.3-1.0	WWTP effluent tank	Sweden	Rainbow trout	Plasma	LOQ: 0.05[d]	[306]			
	n.d.-0.23	Surface water with different wastewater proportion	USA	White perch, Atlantic menhaden, channel catfish, gizzard shad, summer flounder	Plasma	LOD: 0.16[b]	[312]			

Analyte	Concentration [ng/g]	Sampling location	Country	Fish species	Analyzed tissue	Quantification limit [ng/g]	Source	Detected in: Rhine/ Saar fish	Teltow Canal fish	WWTP effluent ponds fish
	n.d.-3.8 (d.w.)	River	USA	Smallmouth bass, largemouth bass, common rudd, rock bass, white bass, white perch, walleye, bowfin, steelhead trout, yellow perch	Fillet	LOQ: 0.035[b]	[300]			
	n.d. (d.w.)	River	USA	Smallmouth bass, largemouth bass, common rudd, rock bass, white bass, white perch, walleye, bowfin/steelhead trout/yellow perch	Liver	LOQ: 0.065[b]	[300]			
2-Hydroxycarbamazepine	n.d.-2.5 (d.w.)	Polluted river sites	USA	Smallmouth bass, largemouth bass, channel catfish, freshwater drum	Fillet	LOQ: 0.1[a]	[301]	-	-	-
	n.d.-10.2 (w.w.)	River	Uruguay	Streaked prochilos, boga, dorado	Fillet	LOQ: 0.063-0.25	[303]			
	n.d.-0.3 (d.w.)	Lagoon	Spain	Golden grey mullet	Fillet	LOQ: 0.2[a]	[302]			
	n.d. (d.w.)	Lagoon	Spain	Black goby	Fillet	LOQ: 0.2[a]	[302]			
	n.d. (d.w.)	Lagoon	Spain	Golden grey mullet	Liver	LOQ: 0.8[a]	[302]			
3-Hydroxycarbamazepine	-	-	-	-	-	-	-	-	-	-
10,11-Dihydro-10-hydroxy-carbamazepine	-	-	-	-	-	-	-	-	-	X
10,11-Dihydroxy-10,11-dihydrocarbamazepine	n.d. (w.w.)	WWTP effluent dominated pond	Czech republic	Common carp	Fillet	LOQ: 0.43-0.70[c]	[287]	-	X	X

Analyte	Concentration [ng/g]	Sampling location	Country	Fish species	Analyzed tissue	Quantification limit [ng/g]	Source	Detected in: Rhine/ Saar fish	Detected in: Teltow Canal fish	Detected in: WWTP effluent ponds fish
	n.d. (w.w.)	WWTP effluent dominated pond	Czech republic	Common carp	Liver	LOQ: 0.86-1.2[c]	[287]			
	n.d.	WWTP effluent dominated pond	Czech republic	Common carp	Plasma	n.a.	[287]			
Acridone	-	-	-	-	-	-	-	-	X	X
Cetirizine	n.d. (w.w.)	WWTP effluent dominated pond	Czech republic	Common carp	Fillet	LOQ: 0.13-0.21[c]	[287]	-	-	-
	0.10 ± 0.04 (w.w.)	WWTP effluent dominated pond	Czech republic	Common carp	Liver	LOQ: 0.049-0.068[c]	[287]			
	n.d.	WWTP effluent dominated pond	Czech republic	Common carp	Plasma	LOQ: 0.098-0.20[c]	[287]			
Chlorothiazide	-	-	-	-	-	-	-	-	-	X
Citalopram	1.3 ± 1.7 (w.w.)	WWTP effluent dominated pond	Czech republic	Common carp	Fillet	LOQ: 0.24-0.40[c]	[287]	-	X	X
	3.1 ± 2.9 (w.w.)	WWTP effluent dominated pond	Czech republic	Common carp	Liver	LOQ: 0.19-0.27[c]	[287]			
	0.21 ± 0.04	WWTP effluent dominated pond	Czech republic	Common carp	Plasma	LOQ: 0.095-0.19[c]	[287]			
	n.d.-2.4 (d.w.)	Polluted river sites	USA	Smallmouth bass, yellow perch, white sucker	Fillet	LOQ: 0.4[a]	[301]			
	0.8 ± 0.1 (d.w.)	River	Spain	Common carp	Whole body	LOQ: 0.41[a]	[284]			

Analyte	Concentration [ng/g]	Sampling location	Country	Fish species	Analyzed tissue	Quantification limit [ng/g]	Source	Detected in: Rhine/ Saar fish	Detected in: Teltow Canal fish	Detected in: WWTP effluent ponds fish
	n.d.-0.4 (d.w.)	River	USA	Smallmouth bass, largemouth bass, common rudd, rock bass, white bass, white perch, walleye, bowfin, steelhead trout, yellow perch	Fillet	LOQ: 0.084[b]	[300]			
	n.d.-3.7 (d.w.)	River	USA	Smallmouth bass, largemouth bass, common rudd, rock bass, white bass, white perch, walleye, bowfin, steelhead trout, yellow perch	Liver	LOQ: 0.249[b]	[300]			
	n.d. (w.w.)	WWTP effluent dominated stream	Czech republic	Brown trout	Fillet	LOQ: 0.062-0.24	[313]			
	n.d.-31 (w.w.)	WWTP effluent dominated stream	Czech republic	Brown trout	Liver	LOQ: 0.090-0.8	[313]			
	n.d.	WWTP effluent dominated stream	Czech republic	Brown trout	Plasma	LOQ: 0.40-1.9	[313]			
	n.d. (w.w.)	WWTP effluent tank	Sweden	Rainbow trout	Fillet	LOQ: 1.4-2.4	[314]			
	12 ± 5 (w.w.)	WWTP effluent tank	Sweden	Rainbow trout	Liver	LOQ: 0.9-2.8	[314]			
	n.d.	WWTP effluent tank	Sweden	Rainbow trout	Plasma	LOQ: 1.4-2.4	[314]			
	<LOQ-2.90 (w.w.)	Cage downstream WWTP	Canada	Fathead minnow	Whole body	LOQ: 0.5[a]	[346]			

Analyte	Concentration [ng/g]	Sampling location	Country	Fish species	Analyzed tissue	Quantification limit [ng/g]	Source	Detected in Rhine/ Saar fish	Teltow Canal fish	WWTP effluent ponds fish
	n.d.-0.4	River	USA	Bull shark	Plasma	LOQ: 0.25	[315]			
	n.d.-0.1625	Cage in wetland receiving WWTP effluent	Canada	Goldfish	Plasma	LOD: 0.08-0.13[a]	[311]			
	0.0392-0.13	Wetland receiving WWTP effluent	Canada	Goldfish	Plasma	LOD: 0.08-0.14[a]	[311]			
Desmethylcitalopram	0.31 ± 0.06 (w.w.)	WWTP effluent dominated pond	Czech republic	Common carp	Fillet	LOQ: 0.15-0.25[c]	[287]	-	X	X
	2.6 ± 1.4 (w.w.)	WWTP effluent dominated pond	Czech republic	Common carp	Liver	LOQ: 0.23-0.32[c]	[287]			
	0.15	WWTP effluent dominated pond	Czech republic	Common carp	Plasma	LOQ: 0.098-0.20[c]	[287]			
	n.d.-2.84 (w.w.)	Cage downstream WWTP	Canada	Fathead minnow	Whole body	LOQ: 0.5[a]	[346]			
Didemethylcitalopram	-	-	-	-	-	-	-	-	-	X
Clopidogrel	n.d.-8 (d.w.)	Polluted river sites	USA	Channel catfish, white sucker	Fillet	LOQ: 0.1[a]	[301]	X	X	X
	n.d.-0.51 (w.w.)	River	Uruguay	Streaked prochilos, boga, dorado	Fillet	LOQ: 0.16-0.37	[303]			
	n.d.-0.2 (d.w.)	Lagoon	Spain	Golden grey mullet	Fillet	LOQ: 0.1[a]	[302]			
	n.d. (d.w.)	Lagoon	Spain	Black goby	Fillet	LOQ: 0.1[a]	[302]			
	n.d. (d.w.)	Lagoon	Spain	Golden grey mullet	Liver	LOQ: 0.6[a]	[302]			
	<LOQ (d.w.)	River	Spain	Common carp	Whole fish	LOQ: 0.13[a]	[284]			
Clopidogrel carboxylic acid	-	-	-	-	-	-	-	-	-	-
Diclofenac	n.d. (w.w.)	WWTP effluent dominated stream	Japan	Crucian carp, common carp	Liver	LOQ:0.83[a]	[285]	X	X	X

Analyte	Concentration [ng/g]	Sampling location	Country	Fish species	Analyzed tissue	Quantification limit [ng/g]	Source	Detected in: Rhine/ Saar fish	Detected in: Teltow Canal fish	Detected in: WWTP effluent ponds fish
	0.15-0.83	WWTP effluent dominated stream	Japan	Crucian carp, common carp	Plasma	LOQ: 0.15[a]	[285]			
	n.d.-0.555 (w.w.)	WWTP effluent dominated stream	Japan	Crucian carp, common carp	Fillet	LOD: 0.042[b]	[305]			
	n.d.-2.86 (w.w.)	WWTP effluent dominated stream	Japan	Crucian carp, common carp	Liver	LOD:0.21[b]	[305]			
	n.d.-1.80	WWTP effluent dominated stream	Japan	Crucian carp, common carp	Plasma	LOD: 0.012[b]	[305]			
	n.d. (w.w.)	WWTP effluent dominated pond	Czech republic	Common carp	Fillet	LOQ: 0.20-0.33[c]	[287]			
	n.d. (w.w.)	WWTP effluent dominated pond	Czech republic	Common carp	Liver	LOQ: 8.4-12[c]	[287]			
	1.7 ± 0.6	WWTP effluent dominated pond	Czech republic	Common carp	Plasma	LOQ: 0.68-1.4[c]	[287]			
	n.d.-0.7 (d.w.)	Polluted river sites	USA	White sucker	Fillet	LOQ: 0.2[a]	[301]			
	n.d.-1.3 (d.w.)	Lagoon	Spain	Golden grey mullet	Fillet	LOQ: 0.4[a]	[302]			
	n.d. (d.w.)	Lagoon	Spain	Black goby	Fillet	LOQ: 0.4[a]	[302]			
	n.d.-2.2 (d.w.)	Lagoon	Spain	Golden grey mullet	Liver	LOQ: 0.4[a]	[302]			
	8.8 ± 0.5 (d.w.)	River	Spain	*Barbus graellsii*	Whole body	LOQ: 1.66[a]	[284]			
	4.1 ± 0.9 (d.w.)	River	Spain	Largemouth bass	Whole body	LOQ: 1.66[a]	[284]			
	2.2-20	WWTP effluent tank	Sweden	Rainbow trout	Plasma	LOQ: 0.1[d]	[306]			
	n.d.-11.930	Surface water with different wastewater proportion	USA	White perch, Atlantic menhaden, channel catfish, gizzard shad, summer flounder	Plasma	LOD: 2.310[b]	[312]			

Analyte	Concentration [ng/g]	Sampling location	Country	Fish species	Analyzed tissue	Quantification limit [ng/g]	Source	Detected in: Rhine/ Saar fish	Detected in: Teltow Canal fish	Detected in: WWTP effluent ponds fish
	n.d.-4.0 (w.w.)	River delta	China	Yellow grouper, orbfish, topmouth culter	Fillet	LOQ: 0.03[a]	[286]			
	1.10-10.76 (d.w.)	River estuary	Malaysia	Sea catfish, croaker	Fillet	LOD: 0.35[a]	[316]			
	n.d.-12	Cage in WWTP effluent	Sweden	Rainbow trout	Plasma	LOD: 3[a]	[347]			
	n.d.-0.48	River, upstream/downstream WWTP	Canada	Pike	Plasma	LOQ: 1[a]	[317]			
Diphenhydramine	6.5-64 (w.w.)	WWTP effluent dominated stream	Japan	Crucian carp, common carp	Liver	LOQ: 0.12[a]	[285]	X	X	X
	0.25-1.8	WWTP effluent dominated stream	Japan	Crucian carp, common carp	Plasma	LOQ: 0.020[a]	[285]			
	0.119-0.744 (w.w.)	WWTP effluent dominated stream	Japan	Crucian carp, common carp	Fillet	LOD: 0.025[b]	[305]			
	1.29-19.8 (w.w.)	WWTP effluent dominated stream	Japan	Crucian carp, common carp	Liver	LOD: 0.028[b]	[305]			
	0.184-1.82	WWTP effluent dominated stream	Japan	Crucian carp, common carp	Plasma	LOD: 0.010[b]	[305]			
	0.14-0.31 (w.w.)	Downstream WWTP	USA	Brown trout	Fillet	LOD: 0.07[b]	[307]			
	n.d.-8.6 (w.w.)	Downstream WWTP	USA	Brown trout	Liver	LOD: 6.0[b]	[307]			
	0.66-1.32 (w.w.)	Effluent dominated stream	USA	*Lepomis*	Fillet	LOD: 0.05[b]	[309]			
	0.08-0.97 (w.w.)	Freshwater pond	USA	Mosquito fish	Whole body	LOD: 0.08[b]	[345]			

Analyte	Concentration [ng/g]	Sampling location	Country	Fish species	Analyzed tissue	Quantification limit [ng/g]	Source	Detected in: Rhine/ Saar fish	Detected in: Teltow Canal fish	Detected in: WWTP effluent ponds fish
	n.d.-0.390	Surface water with different wastewater proportion	USA	White perch, Atlantic menhaden, channel catfish, gizzard shad, summer flounder	Plasma	LOD: 0.130[b]	[312]			
	n.d.-0.5 (d.w)	River	USA	Smallmouth bass, largemouth bass, common rudd, rock bass, white bass, white perch, walleye, bowfin, steelhead trout, yellow perch	Fillet	LOQ: 0.048[b]	[300]			
	n.d.-5.2 (d.w.)	River	USA	Smallmouth bass, largemouth bass, common rudd, rock bass, white bass, white perch, walleye, bowfin, steelhead trout, yellow perch	Liver	LOQ: 0.130[b]	[300]			
	n.d.-0.23	Cage in wetland receiving WWTP effluent	Canada	Goldfish	Plasma	LOD: 0.12-0.24[a]	[311]			
	0.059-0.251	Wetland receiving WWTP effluent	Canada	Goldfish	Plasma	LOD: 0.12-0.24[a]	[311]			
	n.d.-5.3 (w.w.)	River delta	China	Yellow grouper, orbfish, topmouth culter	Fillet	LOQ: 0.2[a]	[286]			
	n.d.-0.07 (w.w.)	Rivers	Germany	Common bream	Fillet	LOD: 0.04[b]	[308]			

Analyte	Concentration [ng/g]	Sampling location	Country	Fish species	Analyzed tissue	Quantification limit [ng/g]	Source	Detected in Rhine/ Saar fish	Teltow Canal fish	WWTP effluent ponds fish
	n.d.-2.83	Urbanization influenced rivers	USA	Common carp, flathead grey mullet, red drum	Plasma	n.a.	[348]			
N-Desmethyl diphenhydramine	-	-	-	-	-	-	-	-	-	X
Fexofenadine	n.d. (w.w.)	WWTP effluent dominated pond	Czech republic	Common carp	Fillet	LOQ: 0.034-0.053[c]	[287]	-	-	-
	n.d. (w.w.)	WWTP effluent dominated pond	Czech republic	Common carp	Liver	LOQ: 0.28-0.39[c]	[287]			
	n.d.	WWTP effluent dominated pond	Czech republic	Common carp	Plasma	LOQ: 0.25-0.5[c]	[287]			
Flecainide	-	-	-	-	-	-	-	X	X	X
Flecainide-meta-O-dealkylated	-	-	-	-	-	-	-	-	-	-
Fluconazole	n.d.-0.18 (w.w.)	River	China	Tilapia, crucian carp, common carp, snakehead fish	Fillet	LOQ: 0.13[a]	[318]	-	-	-
	n.d.-1.91 (w.w.)	River	China	Tilapia, crucian carp, common carp, snakehead fish	Liver	LOQ: 0.61[a]	[318]			
Fluoxetine	n.d. (w.w.)	Downstream WWTP	USA	Brown trout	Fillet	LOD: 5.3[b]	[307]	-	-	X
	18-86 (w.w.)	Downstream WWTP	USA	Brown trout	Liver	LOD: 5.7[b]	[307]			
	n.d.-<LOQ (w.w.)	Cage downstream WWTP	Canada	Fathead minnow	Whole body	LOQ: 0.5[a]	[346]			
	n.d.-<LOQ	River	USA	Bull shark	Plasma	LOQ: 0.25	[315]			

Analyte	Concentration [ng/g]	Sampling location	Country	Fish species	Analyzed tissue	Quantification limit [ng/g]	Source	Detected in: Rhine/ Saar fish	Detected in: Teltow Canal fish	Detected in: WWTP effluent ponds fish
	n.d.-0.302	Cage in wetland receiving WWTP effluent	Canada	Goldfish	Plasma	LOD: 0.3-0.6[a]	[311]			
	0.147-0.4	Wetland receiving WWTP effluent	Canada	Goldfish	Plasma	LOD: 0.3-0.6[a]	[311]			
	n.d-1.02 (w.w.)	Effluent dominated embayment	Canada	Brown bullhead, gizzard shad, white perch	Fillet	LOQ: 0.07[a]	[319]			
	0.11 ± 0.03 (w.w.)	Effluent dominated stream	USA	Bluegill, channel catfish, black crappie	Fillet	LOQ: 0.05[e]	[283]			
	1.34 ± 0.65 (w.w.)	Effluent dominated stream	USA	Bluegill, channel catfish, black crappie	Liver	LOQ: 0.05[e]	[283]			
Norfluoxetine	n.d. (w.w.)	Downstream WWTP	USA	Brown trout	Fillet	LOD: 3.0[b]	[307]	-	-	X
	15-110 (w.w.)	Downstream WWTP	USA	Brown trout	Liver	LOD: 6.7[b]	[307]			
	3.49-5.14 (w.w.)	Effluent dominated stream	USA	*Lepomis*	Fillet	LOD: 2.9[b]	[309]			
	n.d.-1.8 (d.w.)	River	USA	Smallmoufth bass, largemouth bass, common rudd, rock bass, white bass, white perch, walleye, bowfin, steelhead trout, yellow perch	Fillet	LOQ: 0.040[b]	[300]			

Analyte	Concentration [ng/g]	Sampling location	Country	Fish species	Analyzed tissue	Quantification limit [ng/g]	Source	Detected in: Rhine/ Saar fish	Detected in: Teltow Canal fish	Detected in: WWTP effluent ponds fish
	n.d. (d.w.)	River	USA	Smallmouth bass, largemouth bass, common rudd, rock bass, white bass, white perch, walleye, bowfin, steelhead trout, yellow perch	Liver	LOQ: 0.122[b]	[300]			
	n.d.-1.22 (w.w.)	Cage downstream WWTP	Canada	Fathead minnow	Whole body	LOQ: 0.5[a]	[346]			
	n.d.-4.08	River	USA	Bull shark	Plasma	LOQ: 2.5	[315]			
	n.d.-0.879	Cage in wetland receiving WWTP effluent	Canada	Goldfish	Plasma	LOD: 0.365-0.6[a]	[311]			
	0.3-1.1	Wetland receiving WWTP effluent	Canada	Goldfish	Plasma	LOD: 0.365-0.6[a]	[311]			
	n.d.-1.08 (w.w.)	Effluent dominated embayment	Canada	Brown bullhead, gizzard shad, white perch	Fillet	LOQ: 0.14[a]	[319]			
	1.07 ± 0.41 (w.w.)	Effluent dominated stream	USA	Bluegill, channel catfish, black crappie	Fillet	LOQ: 0.05[e]	[283]			
	10.27 ± 5.73 (w.w.)	Effluent dominated stream	USA	Bluegill, channel catfish, black crappie	Liver	LOQ: 0.05[e]	[283]			
	1.10-3.90 (w.w.)	River	Argentina	Thararira, catfish, armored catfish, streaked prochilod	Fillet	LOD: 0.71[b]	[310]			
	n.d.-9.1 (w.w.)	River	Argentina	Thararira, catfish, armored catfish, streaked prochilod	Liver	LOD: 0.71[b]	[310]			

Analyte	Concentration [ng/g]	Sampling location	Country	Fish species	Analyzed tissue	Quantification limit [ng/g]	Source	Detected in: Rhine/ Saar fish	Detected in: Teltow Canal fish	Detected in: WWTP effluent ponds fish
Furosemide	n.d.	Cage in wetland receiving WWTP effluent	Canada	Goldfish	Plasma	LOD: 8-16[a]	[311]	-	-	-
	n.d.	Wetland receiving WWTP effluent	Canada	Goldfish	Plasma	LOD: 8-16[a]	[311]			
Gabapentin lactam	-	-	-	-	-	-	-	X	X	X
Hydrochlorothiazide	n.d.-1.1 (d.w.)	Polluted river sites	USA	Channel catfish, common snook, largemouth bass	Fillet	LOQ: 0.2[a]	[301]	-	X	X
	n.d.-5.98 (w.w.)	River	Uruguay	Streaked prochilos, boga, dorado	Fillet	LOQ: 0.21-0.239	[303]			
	n.d.-10.5 (d.w.)	Lagoon	Spain	Golden grey mullet	Fillet	LOQ: 0.05[a]	[302]			
	n.d.-3.9 (d.w.)	Lagoon	Spain	Black goby	Fillet	LOQ: 0.05[a]	[302]			
	n.d.	Cage in wetland receiving WWTP effluent	Canada	Goldfish	Plasma	LOD: 4-4.8[a]	[311]			
	n.d.	Wetland receiving WWTP effluent	Canada	Goldfish	Plasma	LOD: 4-4.8[a]	[311]			
Lamotrigine	-	-	-	-	-	-	-	-	-	-
Lidocaine	-	-	-	-	-	-	-	-	X	X
Norlidocaine	-	-	-	-	-	-	-	X	X	X
Metoprolol	n.d. (w.w.)	WWTP effluent dominated pond	Czech republic	Common carp	Fillet	LOQ: 0.17-0.23[c]	[287]	-	X	X
	0.42 ± 0.23 (w.w.)	WWTP effluent dominated pond	Czech republic	Common carp	Liver	LOQ: 0.028-0.041[c]	[287]			
	0.13 ± 0.07	WWTP effluent dominated pond	Czech republic	Common carp	Plasma	LOQ: 0.055-0.10[c]	[287]			

Analyte	Concentration [ng/g]	Sampling location	Country	Fish species	Analyzed tissue	Quantification limit [ng/g]	Source	Detected in Rhine/ Saar fish	Teltow Canal fish	WWTP effluent ponds fish
	n.d.-2.4 (w.w.)	River	Uruguay	Streaked prochilos, boga, dorado	Fillet	LOQ: 0.14-0.60	[303]			
	n.d.-0.7 (d.w.)	Lagoon	Spain	Golden grey mullet	Fillet	LOQ: 0.6[a]	[302]			
	n.d. (d.w.)	Lagoon	Spain	Black goby	Fillet	LOQ: 0.6[a]	[302]			
	n.d. (d.w.)	Lagoon	Spain	Golden grey mullet	Liver	LOQ: 2.0[a]	[302]			
O-Desmethyl metoprolol	-	-	-	-	-	-	-	-	-	-
Oxazepam	n.d. (w.w.)	WWTP effluent dominated pond	Czech republic	Common carp	Fillet	LOQ: 0.19-0.33[c]	[287]	-	-	-
	n.d. (w.w.)	WWTP effluent dominated pond	Czech republic	Common carp	Liver	LOQ: 0.76-1.1[c]	[287]			
	n.d.	WWTP effluent dominated pond	Czech republic	Common carp	Plasma	LOQ: 0.20-0.45[c]	[287]			
	0.2-0.7	WWTP effluent tank	Sweden	Rainbow trout	Plasma	LOQ: 0.5[d]	[306]			
	0.389-0.912	Cage in wetland receiving WWTP effluent	Canada	Goldfish	Plasma	LOD: 0.8-1.33[a]	[311]			
	0.417-1.34	Wetland receiving WWTP effluent	Canada	Goldfish	Plasma	LOD: 0.8-1.33[a]	[311]			
	0.39-13 (w.w.)	Stream downstream WWTP	Sweden	European perch	Fillet	LOQ: 0.5	[282]			
Phenytoin	-	-	-	-	-	-	-	-	-	-
Pregabalin lactam	-	-	-	-	-	-	-	-	-	-
Primidone	n.d.-1.05 (d.w.)	River estuary	Malaysia	Sea catfish, croaker	Fillet	LOD: 0.06[a]	[316]	-	-	-
Quetiapine	-	-	-	-	-	-	-	-	-	X
Quetiapine sulfoxide	-	-	-	-	-	-	-	-	-	-
Sertraline	1.0-7.9 (w.w.)	WWTP effluent dominated stream	Japan	Crucian carp, common carp	Liver	LOQ: 0.48[a]	[285]	-	-	X

Analyte	Concentration [ng/g]	Sampling location	Country	Fish species	Analyzed tissue	Quantification limit [ng/g]	Source	Detected in: Rhine/ Saar fish	Detected in: Teltow Canal fish	Detected in: WWTP effluent ponds fish
	0.14-0.51	WWTP effluent dominated stream	Japan	Crucian carp, common carp	Plasma	LOQ: 0.19[a]	[285]			
	0.0348-1.15 (w.w.)	WWTP effluent dominated stream	Japan	Crucian carp, common carp	Fillet	LOD:0.017[b]	[305]			
	0.180-7.86 (w.w.)	WWTP effluent dominated stream	Japan	Crucian carp, common carp	Liver	LOD:0.11[b]	[305]			
	0.0492-0.791	WWTP effluent dominated stream	Japan	Crucian carp, common carp	Plasma	LOD: 0.022[b]	[305]			
	n.d. (w.w.)	Downstream WWTP	USA	Brown trout	Fillet	LOD: 2.1[b]	[307]			
	75-110 (w.w.)	Downstream WWTP	USA	Brown trout	Liver	LOD: 9.6[b]	[307]			
	0.24 ± 0.06 (w.w.)	WWTP effluent dominated pond	Czech republic	Common carp	Fillet	LOQ: 0.094-0.15[c]	[287]			
	1.2 ± 0.5 (w.w.)	WWTP effluent dominated pond	Czech republic	Common carp	Liver	LOQ: 0.075-0.11[c]	[287]			
	n.d.	WWTP effluent dominated pond	Czech republic	Common carp	Plasma	LOQ: 0.22-0.45[c]	[287]			
	n.d.-17.1 (d.w.)	Polluted river sites	USA	White sucker	Fillet	LOQ: 1.1[a]	[301]			
	n.d.-1.2	WWTP effluent tank	Sweden	Rainbow trout	Plasma	LOQ: 0.5[d]	[306]			
	n.d.-1.130	Surface water with different wastewater proportion	USA	White perch, Atlantic menhaden, channel catfish, gizzard shad, summer flounder	Plasma	LOD: 0.99[b]	[312]			
	n.d.-2.4 (w.w.)	WWTP effluent dominated stream	Czech republic	Brown trout	Fillet	LOQ: 0.32-1.2	[313]			

Analyte	Concentration [ng/g]	Sampling location	Country	Fish species	Analyzed tissue	Quantification limit [ng/g]	Source	Detected in: Rhine/ Saar fish	Detected in: Teltow Canal fish	Detected in: WWTP effluent ponds fish
	n.d.-24 (w.w.)	WWTP effluent dominated stream	Czech republic	Brown trout	Liver	LOQ: 0.020-0.18	[313]			
	n.d.-0.77	WWTP effluent dominated stream	Czech republic	Brown trout	Plasma	LOQ: 0.5-0.86	[313]			
	n.d. (w.w.)	WWTP effluent tank	Sweden	Rainbow trout	Fillet	LOQ: 1.3-5.6	[314]			
	4.5 ± 3.2 (w.w.)	WWTP effluent tank	Sweden	Rainbow trout	Liver	LOQ: 1.0-5.2	[314]			
	n.d.	WWTP effluent tank	Sweden	Rainbow trout	Plasma	LOQ: 1.3-5.6	[314]			
	<LOQ-3.83 (w.w.)	Cage downstream WWTP	Canada	Fathead minnow	Whole body	LOQ: 0.5[a]	[346]			
	n.d.-0.48	River	USA	Bull shark	Plasma	LOQ: 0.25	[315]			
	n.d.-0.105	Cage in wetland receiving WWTP effluent	Canada	Goldfish	Plasma	LOD: 0.08-0.16[a]	[311]			
	0.039-0.236	Wetland receiving WWTP effluent	Canada	Goldfish	Plasma	LOD: 0.08-0.16[a]	[311]			
	0.34 ± 0.09 (w.w.)	Effluent dominated stream	USA	Bluegill, channel catfish, black crappie	Fillet	LOQ: 0.05[e]	[283]			
Sitagliptin	-	-	-	-	-	-	-	-	X	X
Sulfamethoxazole	n.d. (w.w.)	WWTP effluent dominated pond	Czech republic	Common carp	Fillet	LOQ: 0.13-0.20[c]	[287]	-	-	-
	0.4 (w.w.)	WWTP effluent dominated pond	Czech republic	Common carp	Liver	LOQ: 0.33-0.61[c]	[287]			
	n.d.	WWTP effluent dominated pond	Czech republic	Common carp	Plasma	LOQ: 0.24-0.47[c]	[287]			

Analyte	Concentration [ng/g]	Sampling location	Country	Fish species	Analyzed tissue	Quantification limit [ng/g]	Source	Detected in: Rhine/ Saar fish	Detected in: Teltow Canal fish	Detected in: WWTP effluent ponds fish
	n.d.-26 (w.w.)	River delta	China	Yellow grouper, orbfish, topmouth culter	Fillet	LOQ: 0.03[a]	[286]			
	n.d.-0.27 (d.w.)	River estuary	Malaysia	Sea catfish, croaker	Fillet	LOD: 0.05[a]	[316]			
	n.d.-1.87 (w.w.)	River	Argentina	Thararira, catfish, armored catfish, streaked prochilod	Fillet	LOD: 1.87[b]	[310]			
	n.d.-2.40 (w.w.)	River	Argentina	Thararira, catfish, armored catfish, streaked prochilod	Liver	LOD: 1.87[b]	[310]			
	n.d.-<LOQ (w.w.)	River	China	Tilapia, crucian carp, common carp, leather catfish, snakehead, grass carp, chub, mud carp, bream	Fillet	LOQ: 0.91[a]	[349]			
	n.d.(w.w.)	River	China	Tilapia, crucian carp, common carp, leather catfish, snakehead, grass carp, chub, mud carp, bream	Liver	-	[349]			
	n.d.-5.50	River	China	Tilapia, crucian carp, common carp, leather catfish, snakehead, grass carp, chub, mud carp, bream	Plasma	LOQ: 4.54[a]	[349]			
N-Acetyl sulfamethoxazole	n.d. (w.w.)	WWTP effluent dominated pond	Czech republic	Common carp	Fillet	LOQ: 0.062-0.11[c]	[287]	-	-	-
	n.d. (w.w.)	WWTP effluent dominated pond	Czech republic	Common carp	Liver	LOQ: 11-20[c]	[287]			

Analyte	Concentration [ng/g]	Sampling location	Country	Fish species	Analyzed tissue	Quantification limit [ng/g]	Source	Detected in: Rhine/ Saar fish	Detected in: Teltow Canal fish	Detected in: WWTP effluent ponds fish
	n.d.	WWTP effluent dominated pond	Czech republic	Common carp	Plasma	LOQ: 0.79-1.5[c]	[287]			
Sulpiride	-	-	-	-	-		-	-	-	-
Telmisartan	n.d. (w.w.)	WWTP effluent dominated pond	Czech republic	Common carp	Fillet	LOQ: 0.14-0.23[c]	[287]	X	-	X
	1.6 ± 0.9 (w.w.)	WWTP effluent dominated pond	Czech republic	Common carp	Liver	LOQ: 0.28-0.39[c]	[287]			
	0.63	WWTP effluent dominated pond	Czech republic	Common carp	Plasma	LOQ: 0.23-0.46[c]	[287]			
Torasemide	-	-	-	-	-	-	-	-	-	-
Hydroxytorasemide	-	-	-	-	-	-	-	-	-	-
Tramadol	n.d. (w.w.)	WWTP effluent dominated pond	Czech republic	Common carp	Fillet	LOQ: 0.71-1.2[c]	[287]	X	X	X
	3.2 ± 1.0 (w.w.)	WWTP effluent dominated pond	Czech republic	Common carp	Liver	LOQ: 0.33-0.54[c]	[287]			
	0.97 ± 0.45	WWTP effluent dominated pond	Czech republic	Common carp	Plasma	LOQ: 0.11-0.22[c]	[287]			
	1.1-1.9	WWTP effluent tank	Sweden	Rainbow trout	Plasma	LOQ: 0.1[d]	[306]			
	n.d.-0.17 (w.w.)	WWTP effluent dominated stream	Czech republic	Brown trout	Fillet	LOQ: 0.085-0.14	[313]			
	n.d.-2.6 (w.w.)	WWTP effluent dominated stream	Czech republic	Brown trout	Liver	LOQ: 0.78-2.5	[313]			
	n.d.	WWTP effluent dominated stream	Czech republic	Brown trout	Plasma	LOQ: 0.40-0.64	[313]			
O-Desmethyl tramadol	-	-	-	-	-	-	-	-	-	X
N-Desmethyl tramadol	-	-	-	-	-	-		X	X	X

Analyte	Concentration [ng/g]	Sampling location	Country	Fish species	Analyzed tissue	Quantification limit [ng/g]	Source	Detected in: Rhine/ Saar fish	Detected in: Teltow Canal fish	Detected in: WWTP effluent ponds fish
N,O-Didesmethyl tramadol	-	-	-	-	-	-	-	-	-	X
Trimethoprim	n.d. (w.w.)	WWTP effluent dominated pond	Czech republic	Common carp	Fillet	LOQ: 0.090-0.14[c]	[287]	-	-	-
	0.4 (w.w.)	WWTP effluent dominated pond	Czech republic	Common carp	Liver	LOQ: 0.090-0.15[c]	[287]			
	n.d.	WWTP effluent dominated pond	Czech republic	Common carp	Plasma	LOQ: 0.058-0.11[c]	[287]			
	n.d.-0.7 (d.w.)	River	USA	Smallmouth bass, largemouth bass, common rudd, rock bass, white bass, white perch, walleye, bowfin, steelhead trout, yellow perch	Fillet	LOQ: 0.162[b]	[300]			
	n.d. (d.w.)	River	USA	Smallmouth bass, largemouth bass, common rudd, rock bass, white bass, white perch, walleye, bowfin, steelhead trout, yellow perch	Liver	LOQ: 0.434[b]	[300]			
	0.04-1.0 (w.w.)	River delta	China	Yellow grouper, orbfish, topmouth culter	Fillet	LOQ: 0.03[a]	[286]			
	n.d.-0.17	River, upstream/downstream WWTP	Canada	Pike	Plasma	LOQ: 1[a]	[317]			

Analyte	Concentration [ng/g]	Sampling location	Country	Fish species	Analyzed tissue	Quantification limit [ng/g]	Source	Detected in: Rhine/ Saar fish	Detected in: Teltow Canal fish	Detected in: WWTP effluent ponds fish
	n.d.-8.80 (w.w.)	River	Argentina	Thararira, catfish, armored catfish, streaked prochilod	Fillet	LOD: 0.45[b]	[310]			
	n.d.-2.80 (w.w.)	River	Argentina	Thararira, catfish, armored catfish, streaked prochilod	Liver	LOD: 0.45[b]	[310]			
	n.d.-0.390 (w.w.)	River	China	Tilapia, crucian carp, common carp, leather catfish, snakehead, grass carp, chub, mud carp, bream	Fillet	LOQ: 0.13[a]	[349]			
	n.d.-2.13 (w.w.)	River	China	Tilapia, crucian carp, common carp, leather catfish, snakehead, grass carp, chub, mud carp, bream	Liver		[349]			
	n.d.-6.13	River	China	Tilapia, crucian carp, common carp, leather catfish, snakehead, grass carp, chub, mud carp, bream	Plasma	LOQ: 4.34[a]	[349]			
3-Desmethyl trimethoprim	-	-	-	-	-	-	-	-	-	-
5-(3,4,5-Trimethoxybenzoyl)-2,4-pyrimidinediamine	-	-	-	-	-	-	-	-	-	-
Valsartan	n.d. (w.w.)	WWTP effluent dominated pond	Czech republic	Common carp	Fillet	LOQ: 0.13-0.22[c]	[287]	-	-	-

Analyte	Concentration [ng/g]	Sampling location	Country	Fish species	Analyzed tissue	Quantification limit [ng/g]	Source	Detected in: Rhine/ Saar fish	Detected in: Teltow Canal fish	Detected in: WWTP effluent ponds fish
	n.d. (w.w.)	WWTP effluent dominated pond	Czech republic	Common carp	Liver	LOQ: 1.3-1.8[c]	[287]			
	n.d.	WWTP effluent dominated pond	Czech republic	Common carp	Plasma	LOQ: 0.80-1.6[c]	[287]			
Valeryl-4-hydroxyvalsartan	-	-	-	-	-	-	-	-	-	-
Valsartanic acid	-	-	-	-	-	-	-	-	-	-
Venlafaxine	0.22 ± 0.02 (w.w.)	WWTP effluent dominated pond	Czech republic	Common carp	Fillet	LOQ: 0.16-0.27[c]	[287]	-	X	X
	1.1 ± 0.4 (w.w.)	WWTP effluent dominated pond	Czech republic	Common carp	Liver	LOQ: 0.18-0.26[c]	[287]			
	0.24 ± 0.08	WWTP effluent dominated pond	Czech republic	Common carp	Plasma	LOQ: 0.10-0.19[c]	[287]			
	n.d.-22.9 (d.w.)	Polluted river sites	USA	Smallmouth bass, largemouth bass, channel catfish, freshwater drum, common snook, spotted brass	Fillet	LOQ: 0.1[a]	[301]			
	n.d.-1.6 (w.w.)	River	Uruguay	Streaked prochilos, boga, dorado	Fillet	LOQ: 0.27-1.25	[303]			
	n.d.-3.1 (d.w.)	Lagoon	Spain	Golden grey mullet	Liver	LOQ: 0.5[a]	[302]			
	0.6 ± 0.02 (d.w.)	River	Spain	Common carp	Whole body	LOQ: 0.15[a]	[284]			

Analyte	Concentration [ng/g]	Sampling location	Country	Fish species	Analyzed tissue	Quantification limit [ng/g]	Source	Detected in: Rhine/ Saar fish	Detected in: Teltow Canal fish	Detected in: WWTP effluent ponds fish
	n.d.-0.7 (d.w.)	River	USA	Smallmouth bass, largemouth bass, common rudd, rock bass, white bass, white perch, walleye, bowfin, steelhead trout, yellow perch	Fillet	LOQ: 0.260[b]	[300]			
	n.d.-56.8 (d.w.)	River	USA	Smallmouth bass, largemouth bass, common rudd, rock bass, white bass, white perch, walleye, bowfin, steelhead trout, yellow perch	Liver	LOQ: 1.10[b]	[300]			
	n.d.-0.56 (w.w.)	WWTP effluent dominated stream	Czech republic	Brown trout	Fillet	LOQ: 0.40-1.5	[313]			
	n.d.-3.5 (w.w.)	WWTP effluent dominated stream	Czech republic	Brown trout	Liver	LOQ: 0.32-1.1	[313]			
	n.d.	WWTP effluent dominated stream	Czech republic	Brown trout	Plasma	LOQ: 0.34-1.7	[313]			
	n.d. (w.w.)	WWTP effluent tank	Sweden	Rainbow trout	Fillet	LOQ: 1.3-2.9	[314]			
	21 $\pm$ 11 (w.w.)	WWTP effluent tank	Sweden	Rainbow trout	Liver	LOQ: 1.0-3.4	[314]			
	2.6 $\pm$ 0.3	WWTP effluent tank	Sweden	Rainbow trout	Plasma	LOQ: 1.3-2.9	[314]			
	n.d.-1.20 (w.w.)	Cage downstream WWTP	Canada	Fathead minnow	Whole body	LOQ: 0.5[a]	[346]			

Analyte	Concentration [ng/g]	Sampling location	Country	Fish species	Analyzed tissue	Quantification limit [ng/g]	Source	Detected in: Rhine/ Saar fish	Detected in: Teltow Canal fish	Detected in: WWTP effluent ponds fish
	<LOQ-0.53	River	USA	Bull shark	Plasma	LOQ: 0.25	[315]			
	n.d.-0.165	Cage in wetland receiving WWTP effluent	Canada	Goldfish	Plasma	LOD: 0.13-0.16[a]	[311]			
	0.039-0.258	Wetland receiving WWTP effluent	Canada	Goldfish	Plasma	LOD: 0.13-0.16[a]	[311]			
N-Desmethyl venlafaxine	n.d.-1.24 (w.w.)	Cage downstream WWTP	Canada	Fathead minnow	Whole body	LOQ: 0.5[a]	[346]	-	-	-
O-Desmethyl venlafaxine	n.d. (w.w.)	WWTP effluent dominated pond	Czech republic	Common carp	Fillet	LOQ: 0.18-0.25[c]	[287]	-	X	X
	0.74 ± 0.48 (w.w.)	WWTP effluent dominated pond	Czech republic	Common carp	Liver	LOQ: 0.15-0.23[c]	[287]			
	0.21 ± 0.09	WWTP effluent dominated pond	Czech republic	Common carp	Plasma	LOQ: 0.083-0.15[c]	[287]			
	n.d.-<LOQ (w.w.)	Cage downstream WWTP	Canada	Fathead minnow	Whole body	LOQ: 0.5[a]	[346]			
N,O-Desmethyl venlafaxine	-	-	-	-	-	-	-	-	-	-
Xipamide	-	-	-	-	-	-	-	-	-	-

[a] Determination based on signal to noise

[b] Determination based on standard deviation between matrix spike replicates

[c] Determined as the lowest concentration providing relative standard deviation inferior to a threshold

[d] Determination based on standard deviation between blanks

[e] Determination based on signal to noise on 2 MRM transitions

Table C.3: Sum formulas, suppliers and multiple reaction monitoring parameters of the analytes

Name	Formula	Supplier	Retention time [min]	MRM 1 (Quantification)	MRM 2 (Qualification)	DP [V]	CE [eV]	CXP [V]	Polarity
Amisulpride	$C_{17}H_{27}N_3O_4S$	TRC	5.49	370.2/242	370.2/196	106	39/59	14/12	Positive
Bezafibrate	$C_{19}H_{20}ClNO_4$	Sigma Aldrich	11.24	360.1/274.1	360.1/154	-65	-22/-36	-17/-9	Negative
Bicalutamide	$C_{18}H_{14}F_4N_2O_4S$	TRC	11.81	429.1/255	429.1/185	-55	-22/-50	-13/-9	Negative
Candesartan	$C_{24}H_{20}N_6O_3$	TRC	10.27	441.2/263.2	441.2/207.2	51	17/35	16/12	Positive
Carbamazepine	$C_{15}H_{12}N_2O$	Sigma Aldrich	9.33	237.1/194	237.1/179.1	71	27/49	16/12	Positive
2-Hydroxycarbamazepine	$C_{15}H_{12}N_2O_2$	Novartis	7.66	253.1/210.2	253.1/208	71	29/35	12/18	Positive
3-Hydroxycarbamazepine	$C_{15}H_{12}N_2O_2$	Novartis	8.11	253.1/210.1	253.1/167	66	27/51	14/10	Positive
10,11-Dihydro-10-hydroxy-carbamazepine	$C_{15}H_{14}N_2O_2$	Novartis	7.39	255.2/194.1	255.2/179.1	46	27/52	14/14	Positive
10,11-Dihydroxy-10,11-dihydrocarbamazepine	$C_{15}H_{14}N_2O_3$	TRC	6.85	271/180	271/236	40	45/19	12/6	Positive
Acridone	$C_{13}H_9NO$	Th. Geyer	8.76	196/167.1	196/139.1	96	43/71	30/22	Positive
Cetirizine	$C_{21}H_{25}ClN_2O_3$	TRC	9.15	389.1/166.1	389.1/201.1	55	60/30	10/10	Positive
Chlorothiazide	$C_7H_6ClN_3O_4S_2$	Sigma Aldrich	5.60	294/179	294/214	-80	-62/-40	-10/-4	Negative
Citalopram	$C_{20}H_{21}FN_2O$	Labmix24	7.53	325.2/262.1	325.2/109.1	85	27/37	10/10	Positive
Desmethylcitalopram	$C_{19}H_{19}FN_2O$	TRC	7.43	311.1/262.1	311.1/109.1	45	26/32	10/10	Positive
Didemethylcitalopram	$C_{18}H_{17}FN_2O$	TRC	7.31	297.1/116	297.1/109	40	30/30	6/6	Positive
Clopidogrel	$C_{16}H_{16}ClNO_2S$	TRC	14.89	322.1/212	322.1/184	31	23/31	14/12	Positive
Clopidogrel carboxylic acid	$C_{15}H_{14}ClNO_2S$	TRC	6.94	308/198	308/141	66	23/47	12/22	Positive
Diclofenac	$C_{14}H_{11}Cl_2NO_2$	Sigma Aldrich	12.88	296/215	n.a.	46	27	15	Positive
Diphenhydramine	$C_{17}H_{21}NO$	TRC	7.42	256.2/167	256.2/152	20	20/50	5/8	Positive
N-Desmethyl diphenhydramine	$C_{16}H_{19}NO$	TRC	7.27	242/167	242/152	30	20/50	10/10	Positive
Fexofenadine	$C_{32}H_{39}NO_4$	TRC	8.32	502.3/466.3	502.3/171.1	80	38/57	5/5	Positive
Flecainide	$C_{17}H_{20}F_6N_2O_3$	TRC	7.67	415.2/398.1	415.2/301	80	35/50	10/10	Positive
Flecainide-meta-O-dealkylated	$C_{15}H_{19}F_3N_2O_3$	TRC	5.82	333.1/316.1	333.1/219.1	60	28/40	8/8	Positive
Fluconazole	$C_{13}H_{12}F_2N_6O$	TRC	6.78	307.1/238.1	307.1/220.1	70	20/25	20/15	Positive
Fluoxetine	$C_{17}H_{18}F_3NO$	Dr. Ehrenstorfer	8.52	310/44	310/148	45	30/14	10/10	Positive
Norfluoxetine	$C_{16}H_{16}F_3NO$	TRC	8.36	296/134	n.a.	40	11	10	Positive
Furosemide	$C_{12}H_{11}ClN_2O_5S$	Sigma Aldrich	9.29	329/205	329/285	-90	-30/-20	-9/-13	Negative

Name	Formula	Supplier	Retention time [min]	MRM 1 (Quantification)	MRM 2 (Qualification)	DP [V]	CE [eV]	CXP [V]	Polarity
Gabapentin lactam	$C_9H_{15}NO$	Sigma Aldrich	8.07	154.1/95	154.1/67	80	30/40	12/12	Positive
Hydrochlorothiazide	$C_7H_8ClN_3O_4S_2$	TRC	5.83	296/268.9	296/205	-120	-26/-32	-13/-11	Negative
Lamotrigine	$C_9H_7Cl_2N_5$	TCI	5.86	256/211	256/157	80	38/45	10/10	Positive
Lidocaine	$C_{14}H_{22}N_2O$	TRC	5.64	235.2/86.1	235.2/58.1	80	23/53	14/2	Positive
Norlidocaine	$C_{12}H_{18}N_2O$	TRC	5.31	207.1/58	n.a.	35	30	8	Positive
Metoprolol	$C_{15}H_{25}NO_3$	Sigma Aldrich	5.95	268/116	268/74	75	27/35	10/11	Positive
O-Desmethyl metoprolol	$C_{14}H_{23}NO_3$	TRC	5.08	254.2/116	254.2/177	70	25/25	8/10	Positive
Oxazepam	$C_{15}H_{11}ClN_2O_2$	Sigma Aldrich	9.73	287.1/241	287.1/104	61	47/81	8/6	Positive
Phenytoin	$C_{15}H_{12}N_2O_2$	Sigma Aldrich	9.40	251/102	n.a.	-60	-36	-8	Negative
Pregabalin lactam	$C_8H_{15}NO$	TRC	8.34	142.1/55.1	142.1/41	45	37/45	10/10	Positive
Primidone	$C_{12}H_{14}N_2O_2$	Sigma Aldrich	6.64	219/162	n.a.	40	16	13	Positive
Quetiapine	$C_{21}H_{25}N_3O_2S$	TCI	7.42	384.2/253.2	384.2/221.3	80	30/60	11/15	Positive
Quetiapine sulfoxide	$C_{21}H_{25}N_3O_3S$	TRC	6.20	400/221	400/269	90	50/29	5/6	Positive
Sertraline	$C_{17}H_{17}Cl_2N$	Sigma Aldrich	8.56	306.3/275.1	306.3/159	63	20/37	7/13	Positive
Sitagliptin	$C_{16}H_{15}F_6N_5O$	TRC	6.36	408.1/235.1	408.1/174	51	29/33	38/24	Positive
Sulfamethoxazole	$C_{10}H_{11}N_3O_3S$	Sigma Aldrich	7.71	254.1/156	254.1/188	66	23/21	12/14	Positive
N-Acetyl sulfamethoxazole	$C_{12}H_{13}N_3O_4S$	EAWAG	7.91	296.1/198	296.1/134	81	25/35	14/12	Positive
Sulpiride	$C_{15}H_{23}N_3O_4S$	Sigma Aldrich	4.91	342.2/214	342.2/112.1	70	45/35	10/8	Positive
Telmisartan	$C_{33}H_{30}N_4O_2$	TRC	10.27	515.2/276.1	515.2/497.2	181	61/45	26/22	Positive
Torasemide	$C_{16}H_{20}N_4O_3S$	TRC	7.89	349.1/264.1	349.1/290.1	60	25/20	8/8	Positive
Hydroxytorasemide	$C_{16}H_{20}N_4O_4S$	TRC	5.88	365.1/280.1	365.1/306.1	50	25/20	8/8	Positive
Tramadol	$C_{16}H_{25}NO_2$	Fluka	5.98	264.2/58	n.a.	46	45	4	Positive
O-Desmethyl tramadol	$C_{15}H_{23}NO_2$	Sigma Aldrich	5.23	250.1/58	n.a.	45	20	11	Positive
N-Desmethyl tramadol	$C_{15}H_{23}NO_2$	TRC	6.15	250.1/44	n.a.	45	20	11	Positive
N,O-Didesmethyl tramadol	$C_{14}H_{21}NO_2$	TRC	5.27	236.1/44	n.a.	44	20	8	Positive
Trimethoprim	$C_{14}H_{18}N_4O_3$	Sigma Aldrich	5.34	291.1/261.1	291.1/230.1	86	35/33	10/11	Positive
3-Desmethyl trimethoprim	$C_{13}H_{16}N_4O_3$	TRC	5.02	277.1/261.1	277.1/123.1	86	38/51	15/10	Positive
5-(3.4,5-Trimethoxybenzoyl)-2,4-pyrimidinediamine	$C_{14}H_{16}N_4O_4$	TRC	6.22	305.1/137	305.1/244.1	80	35/35	7/6	Positive

Name	Formula	Supplier	Retention time [min]	MRM 1 (Quantification)	MRM 2 (Qualification)	DP [V]	CE [eV]	CXP [V]	Polarity
Valsartan	$C_{24}H_{29}N_5O_3$	TRC	11.80	436.2/235.1	436.2/207.1	111	27/35	12/16	Positive
Valeryl-4-hydroxyvalsartan	$C_{24}H_{29}N_5O_4$	TRC	9.20	450.5/350	450.5/179	-75	-26/-40	-3/-8	Negative
Valsartanic acid	$C_{14}H_{10}N_4O_2$	TRC	8.17	267.1/206.1	267.1/151.1	80	17/57	10/10	Positive
Venlafaxine	$C_{17}H_{27}NO_2$	Promochem	6.67	278.2/58	278.2/121.1	36	43/28	8/8	Positive
N-Desmethyl venlafaxine	$C_{16}H_{25}NO_2$	Campro Scientific	6.53	264.1/44	264.1/121.1	36	55/37	11/10	Positive
O-Desmethyl venlafaxine	$C_{16}H_{25}NO_2$	Promochem	5.64	264.1/58	n.a.	56	45	8	Positive
N,O-Desmethyl venlafaxine	$C_{15}H_{23}NO_2$	Campro Scientific	5.59	250.2/44.2	250.2/132.9	36	32/31	10/10	Positive
Xipamide	$C_{15}H_{15}ClN_2O_4S$	TRC	11.07	353.1/274.1	353.1/127	-35	-30/-26	-4/-3	Negative

Table C.4: Suppliers and multiple reaction monitoring parameters of the surrogates

Name	Supplier	Retention time [min]	MRM	DP [V]	CE [eV]	CXP [V]	Polarity
Amisulpride-d_5	TRC	5.49	375.2/242	106	39	10	Positive
Bezafibrate-d_4	TRC	11.24	364.1/158	-65	-38	-11	Negative
Bicalutamide-d_4	TRC	11.81	433.1/185	-55	-50	-8	Negative
Candesartan-d_5	TRC	10.27	446.2/268	66	17	14	Positive
Carbamazepine-$^{15}N,^{13}C$	Campro Scientific	9.33	239/192	61	29	12	Positive
10,11-Dihydro-10-hydroxy-carbamazepine-d_3	TLC	7.39	258.2/197.2	58	29	17	Positive
Cetirizine-d_8	TRC	9.15	397.2/166.1	55	55	10	Positive
Chlorothiazide-$^{13}C,^{15}N_2$	TRC	5.60	297/216	-90	-40	-10	Negative
Citalopram-d_6	TRC	7.53	331.2/109.1	60	37	10	Negative
Didemethylcitalopram-d_6	TRC	7.31	303.2/266.1	60	22	6	Positive
Clopidogrel-d_4	TRC	14.89	326.1/216.1	31	23	10	Positive
Clopidogrel carboxylic acid-d_4	TRC	6.94	312/202	60	25	15	Positive
Diclofenac-d_4	Dr Ehrenstorfer	12.88	300/219	46	27	15	Positive
Diphenhydramine-d_6	TRC	7.42	262.2/152	30	55	8	Positive
N-Desmethyl diphenhydramine-d_3	TRC	7.27	245.2/167	30	20	8	Positive
Fexofenadine-d_6	TRC	8.32	508.3/472.3	80	40	5	Positive
Flecainide-d_3	TRC	7.67	418.2/401.2	70	35	10	Positive
Fluconazole-d_4	TRC	6.78	311.1/223.1	70	25	17	Positive
Fluoxetine-d_5	Sigma-Aldrich	8.52	315/44	46	30	10	Positive
Norfluoxetine-d_5	Santa Cruz	8.36	301/139	31	11	10	Positive
Furosemide-d_5	TRC	9.29	334/206	-125	-34	-11	Negative
Gabapentin lactam-d_6	TRC	8.07	160.3/101.1	81	33	8	Positive
Hydrochlorothiazide-$^{13}C,d_2$	TRC	5.83	299/269.9	-130	-28	-13	Negative
Lamotrigine-$^{13}C,^{15}N_4$	Sigma Aldrich	5.86	261/46.1	86	79	4	Positive
Lidocaine-ethyl-d_{10}	TRC	5.64	245.2/96.1	96	23	12	Positive
Norlidocaine-d_5	TRC	5.31	212.3/63.3	40	30	8	Positive
Metoprolol-d_7	Campro Scientific	5.95	275/123	80	27	16	Positive

Name	Supplier	Retention time [min]	MRM 1	DP [V]	CE [eV]	CXP [V]	Polarity
O-Desmethyl metoprolol-d_5	TRC	5.08	259.2/182.2	70	28	10	Positive
Oxazepam-d_5	Sigma Aldrich	9.73	292.1/246	81	47	20	Positive
Primidone-d_5	TRC	6.64	224/167.1	56	17	14	Positive
Quetiapine-d_8	TRC	7.42	392/258	70	35	20	Positive
Sertraline-d_3	TRC	8.56	309/159	36	33	14	Positive
Sitagliptin-d_4	TRC	6.36	412.1/239.1	26	27	14	Positive
Sulfamethoxazole-d_4	TRC	7.71	258/160	66	23	12	Positive
N-Acetyl sulfamethoxazole-d_4	TRC	7.91	300/202	81	23	18	Positive
Sulpiride-d_3	TRC	4.91	345.4/112.1	80	36	8	Positive
Telmisartan-d_3	TRC	10.27	518.3/500.2	171	47	20	Positive
Torasemide-d_7	TRC	7.89	356.1/264.1	60	25	8	Positive
Hydroxytorasemide-d_7	TRC	5.88	372.1/306.1	55	20	8	Positive
Tramadol-d_6	TRC	5.98	270.2/64	61	43	12	Positive
O-Desmethyl tramadol-d_6	TRC	5.23	256.2/64	41	84	8	Positive
N-Desmethyl tramadol-d_3	TRC	6.05	253.2/47	45	20	11	Positive
N,O-Didesmethyl tramadol-d_3	TRC	5.27	239.1/47	47	20	9	Positive
Trimethoprim-d_3	Sigma Aldrich	5.34	294/123.1	90	33	10	Positive
Valsartan-d_3	TRC	11.8	439.2/207.1	111	35	16	Positive
Valsartanic acid-d_4	TRC	8.17	271.1/210.1	80	17	10	Positive
Venlafaxine-d_6	TRC	6.67	284.2/58	41	43	4	Positive
N-Desmethyl venlafaxine-d_3	TRC	6.53	267.2/47	44	38	9	Positive
N,O-Desmethyl venlafaxine-d_3	TRC	5.59	253.2/47	48	47	8	Positive
Xipamide-d_6	TRC	11.07	359.1/78	-35	-30	-4	Negative

Table C.5: Glucuronide standard suppliers and multiple reaction monitoring parameters

Name	CAS	Supplier	Retention time [min]	MRM	DP [V]	CE [eV]	CXP [V]	Polarity
Diphenhydramine N-β-D-glucuronide	137908-78-2	TRC	7.61	432.5/167.2	88	36	13	Positive
Lamotrigine N-2-β-D-glucuronide	133310-19-7	Santa Cruz	5.28	432/256	32	70	6	Positive
(R)-Naproxen Acyl-β-D-glucuronide	112828-15-6	TRC	9.28	405.2/185	-44	-23	-27	Negative
4-Acetaminophen sulfate potassium salt	32113-41-0	TRC	5.22	229.8/149.9	-43	-24	-24	Negative
p-Acetamidophenyl β-D glucuronide	120595-80-4	Sigma Aldrich	4.79	326.1/113	-60	-19	-7	Negative
O-Desmethyl venlafaxine β-D-glucuronide	1021933-98-1	Santa Cruz	5.63	438/113	-79	-28	-6	Negative

Table C.6: Parameters of the LC-MS method

LC	Agilent 1260 (Waldbronn, Germany)
Column guard	Zorbax Eclipse SB-C8 (2.1 × 12.5 mm, 5 µm, Agilent)
Column	Zorbax Eclipse Plus C18 column (2.1 × 150 mm, 3.5 µm, Agilent)
Injection volume	80 µL
Flow rate	300 µL/min
Mobile phase A	0.1% acetic acid
Mobile phase B	Acetonitrile
Gradient	0-1 min, 0% B
	1-2 min 0-20% B
	2-16 min, 20-100% B
	16-19 min, 100% B
	19-25 min, 0-100% B
Column temperature	25 °C
MS	Triple quadrupole (API 6500 QTrap, SCIEX, Darmstadt, Germany)
Ion source	Ion drive ™
Ionization mode	Electrospray with polarity switch
Curtain gas	45 psi
Ion source gas 1	45 psi
Ion source gas 2	40 psi
Source temperature	450 °C
Entrance potential	10 V (positive mode)/-10 V (negative mode)
Ion spray voltage	5500 V (positive mode)/-4500 V (negative mode)
MRM mode	Advanced scheduled MRM
Target scan time	0.2 s (positive mode)
	0.2 s (negative mode)
Post-LC divert valve to waste	0.0-2.0 min
	17-25 min
MS data acquisition software	Analyst 1.6.2

Table C.7: Analytes with their corresponding internal standard

Analytes	Internal standard
Amisulpride	Amisulpride-d_5
Bezafibrate	Bezafibrate-d_4
Bicalutamide	Bicalutamide-d_4
Candesartan	Candesartan-d_5
Carbamazepine	Carbamazepine-$^{15}N,^{13}C$
2-Hydroxycarbamazepine	10,11-Dihydro-10-hydroxy-carbamazepine-d_3
3-Hydroxycarbamazepine	10,11-Dihydro-10-hydroxy-carbamazepine-d_3
10,11-Dihydro-10-hydroxy-carbamazepine	10,11-Dihydro-10-hydroxy-carbamazepine-d_3
10,11-Dihydroxy-10,11-dihydrocarbamazepine	10,11-Dihydro-10-hydroxy-carbamazepine-d_3
Acridone	Carbamazepine-$^{15}N,^{13}C$
Cetirizine	Cetirizine-d_8
Chlorothiazide	Chlorothiazide-$^{13}C,^{15}N_2$
Citalopram	Citalopram-d_6
Desmethylcitalopram	Citalopram-d_6
Didemethylcitalopram	Didemethylcitalopram-d_6
Clopidogrel	Clopidogrel-d_4
Clopidogrel carboxylic acid	Clopidogrel carboxylic acid-d_4
Diclofenac	Diclofenac-d_4
Diphenhydramine	Diphenhydramine-d_6
N-Desmethyl diphenhydramine	N-Desmethyl diphenhydramine-d_3
Fexofenadine	Fexofenadine-d_6
Flecainide	Flecainide-d_3
Flecainide-meta-O-dealkylated	Flecainide-d_3
Fluconazole	Fluconazole-d_4
Fluoxetine	Fluoxetine-d_5
Norfluoxetine	Norfluoxetine-d_5
Furosemide	Furosemide-d_5
Gabapentin lactam	Gabapentin lactam-d_6
Hydrochlorothiazide	Hydrochlorothiazide-$^{13}C,d_2$
Lamotrigine	Lamotrigine-$^{13}C,^{15}N_4$
Lidocaine	Lidocaine-ethyl-d_{10}
Norlidocaine	Norlidocaine-d_5
Metoprolol	Metoprolol-d_7
O-Desmethyl metoprolol	O-Desmethyl metoprolol-d_5
Oxazepam	Oxazepam-d_5
Phenytoin	Primidone-d_5
Pregabalin lactam	Gabapentin lactam-d_6
Primidone	Primidone-d_5
Quetiapine	Quetiapine-d_8
Quetiapine sulfoxide	Carbamazepine-$^{15}N,^{13}C$
Sertraline	Sertraline-d_3
Sitagliptin	Sitagliptin-d_4
Sulfamethoxazole	Sulfamethoxazole-d_4
N-Acetyl sulfamethoxazole	Lamotrigine-$^{13}C,^{15}N_4$
Sulpiride	Sulpiride-d_3
Telmisartan	Telmisartan-d_3
Torasemide	Torasemide-d_7
Hydroxytorasemide	Hydroxytorasemide-d_7

Analytes	Internal standard
Tramadol	Tramadol-d_6
O-Desmethyl tramadol	O-Desmethyl tramadol-d_6
N-Desmethyl tramadol	N-Desmethyl tramadol-d_3
N,O-Didesmethyl tramadol	N,O-Didesmethyl tramadol-d_3
Trimethoprim	Trimethoprim-d_3
3-Desmethyl trimethoprim	Oxazepam-d_5
5-(3,4,5-Trimethoxybenzoyl)-2,4-pyrimidinediamine	Clopidogrel carboxylic acid-d_4
Valsartan	Valsartan-d_3
Valeryl-4-hydroxyvalsartan	Bezafibrate-d_4
Valsartanic acid	Valsartanic acid-d_4
Venlafaxine	Venlafaxine-d_6
N-Desmethyl venlafaxine	N-Desmethyl venlafaxine-d_3
O-Desmethyl venlafaxine	N,O-Desmethyl venlafaxine-d_3
N,O-Desmethyl venlafaxine	N,O-Desmethyl venlafaxine-d_3
Xipamide	Xipamide-d_6

Table C.8: Lipid content of the analyzed samples

Sample location	Matrix	Lipid content [%, dry weight basis]
Koblenz 2014	Fillet	27.6[a]
	Liver	39.8
Koblenz 2015	Fillet	29.2[a]
	Liver	56.6
Koblenz 2016	Fillet	27.6[a]
	Liver	46.7
Bimmen 2015	Fillet	19.2[a]
	Liver	35.8
Rehlingen 2015	Fillet	24.6[a]
	Liver	20.6
Pond fed by WWTP 1	Fillet	3.88
	Liver	7.26
Pond fed by WWTP 2	Fillet	4.21
	Liver	11.04
Pond fed by WWTP 3	Fillet	5.77
	Liver	7.66
Pond fed by WWTP 4	Fillet	2.82
	Liver	9.19
Pond fed by WWTP 5	Fillet	10.89
	Liver	11.21
Teltow canal U1[b]	Fillet	4.79
Teltow canal U2[b]	Fillet	8.63
Teltow canal U3[b]	Fillet	5.91
Teltow canal U4[b]	Fillet	5.00
Teltow canal U5[b]	Fillet	10.46
Teltow canal U6[b]	Fillet	5.72
Teltow canal U7[b]	Fillet	6.51
Teltow canal U8[b]	Fillet	6.74
Teltow canal U9[b]	Fillet	11.09
Teltow canal U10[b]	Fillet	9.82
Teltow canal U11[b]	Fillet	7.45

[a] Determined by the German Environmental Specimen Bank then converted in dry weight for comparability with the values determined in our laboratory

[b] Due to an insufficient amount of samples, lipid content could not be determined for liver samples

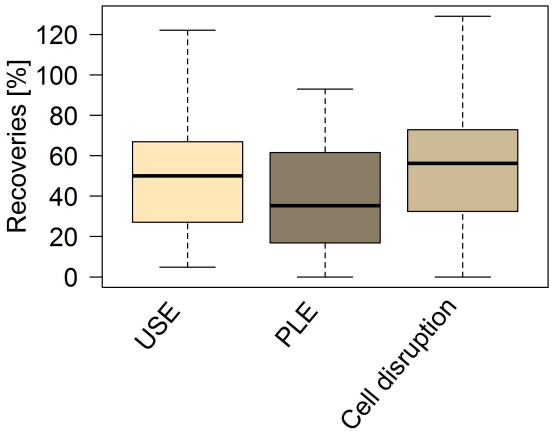

Figure C.1: Recoveries over the whole method according to the extraction technique. USE: ultrasound extraction, PLE: pressurized liquid extraction

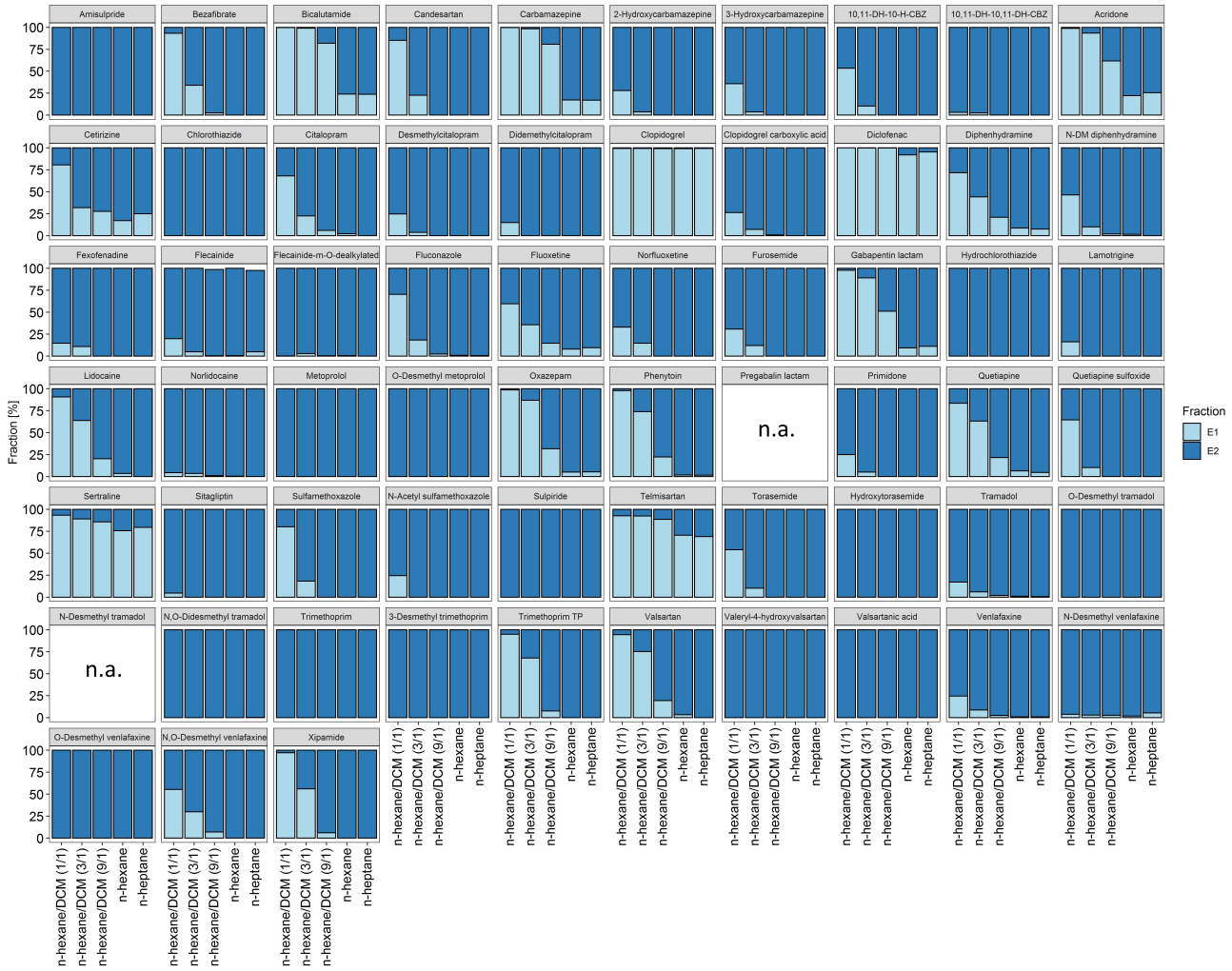

Figure C.2: Distribution of the analytes between both extraction solvents according to the composition of the first extraction solvent. E1: Proportion of the analyte extracted by the first solvent, E2: proportion of analyte extracted by the second solvent. 10,11-DH-10-H-CBZ: 10,11-Dihydro-10-hydroxy-carbamazepine, 10,11-DH-10,11-DH-CBZ: 10,11-Dihydroxy-10,11-dihydrocarbamazepine, N-DM diphenhydramine: N-desmethyldiphenhydramine, Trimethoprim TP: 5-(3,4,5-Trimethoxybenzoyl)-2,4-pyrimidinediamine.

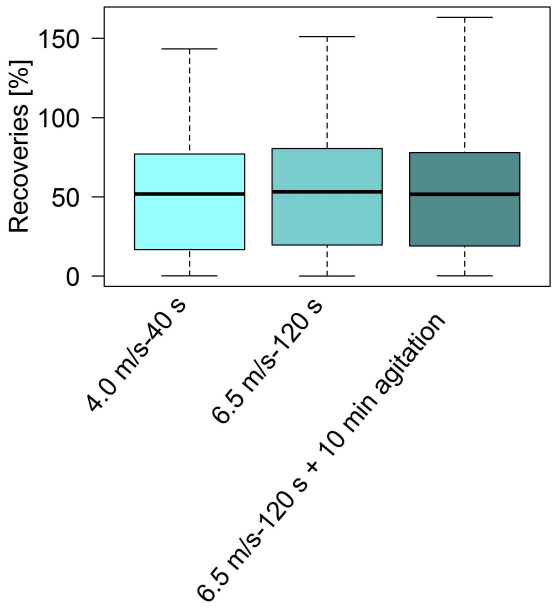

Figure C.3: Recoveries over the whole analytical method according to extraction times for the cell disruption procedure for the investigated analytes

Table C.9: Recoveries over the extraction procedure with their 95% confidence interval (n=3).

Analytes	Recoveries [%]
Amisulpride	103 ± 7
Bezafibrate	87 ± 26
Bicalutamide	104 ± 3
Candesartan	106 ± 18
Carbamazepine	101 ± 4
2-Hydroxycarbamazepine	n.a.
3-Hydroxycarbamazepine	70 ± 6
10,11-Dihydro-10-hydroxy-carbamazepine	74 ± 13
10,11-Dihydroxy-10,11-dihydrocarbamazepine	67 ± 24
Acridone	n.a.
Cetirizine	90 ± 15
Citalopram	120 ± 5
Desmethylcitalopram	77 ± 11
Didemethylcitalopram	68 ± 30
Clopidogrel	79 ± 10
Clopidogrel carboxylic acid	71 ± 22
Diclofenac	110 ± 9
Diphenhydramine	97 ± 8
N-Desmethyl diphenhydramine	100 ± 17
Fexofenadine	n.a.
Flecainide	87 ± 7
Flecainide-meta-O-dealkylated	108 ± 11
Fluconazole	103 ± 12
Fluoxetine	65 ± 10
Norfluoxetine	n.a.

Analytes	Recoveries (n=3) [%]
Furosemide	69 ± 28
Gabapentin lactam	100 ± 15
Hydrochlorothiazide	111 ± 5
Chlorothiazide	113 ± 17
Lamotrigine	n.a.
Lidocaine	91 ± 16
Norlidocaine	102 ± 32
Metoprolol	97 ± 45
O-Desmethyl metoprolol	n.a.
Oxazepam	94 ± 8
Phenytoin	n.a.
Primidone	131 ± 32
Quetiapine	86 ± 27
Quetiapine sulfoxide	129 ± 13
Sertraline	n.a.
Sitagliptin	108 ± 13
Sulfamethoxazole	n.a.
N-Acetyl sulfamethoxazole	n.a.
Sulpiride	96 ± 11
Telmisartan	n.a.
Torasemide	95 ± 19
Hydroxytorasemide	73 ± 21
Tramadol	97 ± 12
O-Desmethyl tramadol	98 ± 27
N,O-Didesmethyl tramadol	91 ± 20
Trimethoprim	n.a.
3-Desmethyl trimethoprim	n.a.
5-(3,4,5 Trimethoxybenzoyl)-2,4-pyrimidinediamine	n.a.
Valsartan	n.a.
Valeryl-4-hydroxyvalsartan	n.a.
Valsartanic acid	n.a.
Venlafaxine	102 ± 10
N-Desmethyl venlafaxine	101 ± 23
O-Desmethyl venlafaxine	n.a.
N,O-Desmethyl venlafaxine	n.a.
Xipamide	88 ± 15

Table C.10: Analytes recoveries over the RAM clean-up with their 95% confidence interval (n=3)

Analytes	Recoveries (n=3) [%]
Amisulpride	90 ± 10
Bezafibrate	100 ± 10
Bicalutamide	97 ± 9
Candesartan	91 ± 8
Carbamazepine	97 ± 7
2-Hydroxycarbamazepine	84 ± 3
3-Hydroxycarbamazepine	80 ± 30
10,11-Dihydro-10-hydroxy-carbamazepine	98 ± 4
10,11-Dihydroxy-10,11-dihydrocarbamazepine	86 ± 7
Acridone	96 ± 6
Cetirizine	240 ± 40
Chlorothiazide	25 ± 4
Citalopram	90 ± 10
Desmethylcitalopram	90 ± 20
Didemethylcitalopram	92 ± 7
Clopidogrel	101 ± 8
Clopidogrel carboxylic acid	99 ± 5
Diclofenac	110 ± 20
Diphenhydramine	93 ± 9
N-Desmethyl diphenhydramine	94 ± 6
Fexofenadine	93 ± 7
Flecainide	100 ± 20
Flecainide-meta-O-dealkylated	110 ± 20
Fluconazole	100 ± 10
Fluoxetine	91 ± 3
Norfluoxetine	90 ± 30
Furosemide	90 ± 10
Gabapentin lactam	102 ± 5
Hydrochlorothiazide	56 ± 10
Lamotrigine	86 ± 6
Lidocaine	91 ± 7
Norlidocaine	60 ± 10
Metoprolol	94 ± 6
O-Desmethyl metoprolol	68 ± 6
Oxazepam	104 ± 5
Phenytoin	90 ± 20
Pregabalin lactam	90 ± 20
Primidone	80 ± 9
Quetiapine	110 ± 70
Quetiapine sulfoxide	9 ± 5
Sertraline	85 ± 10
Sitagliptin	95 ± 9
Sulfamethoxazole	92 ± 2
N-Acetyl sulfamethoxazole	82 ± 5
Sulpiride	100 ± 10
Telmisartan	110 ± 60
Torasemide	99 ± 9
Hydroxytorasemide	100 ± 10

Analytes	Recoveries (n=3) [%]
Tramadol	92 ± 10
O-Desmethyl tramadol	90 ± 4
N-Desmethyl tramadol	90 ± 10
N,O-Didesmethyl tramadol	95 ± 7
Trimethoprim	95 ± 2
3-Desmethyl trimethoprim	110 ± 10
5-(3,4,5-Trimethoxybenzoyl)-2,4-pyrimidinediamine	100 ± 10
Valsartan	92 ± 8
Valeryl-4-hydroxyvalsartan	100 ± 10
Valsartanic acid	110 ± 20
Venlafaxine	88 ± 5
N-Desmethyl venlafaxine	93 ± 5
O-Desmethyl venlafaxine	93 ± 4
N,O-Desmethyl venlafaxine	99 ± 9
Xipamide	90 ± 10

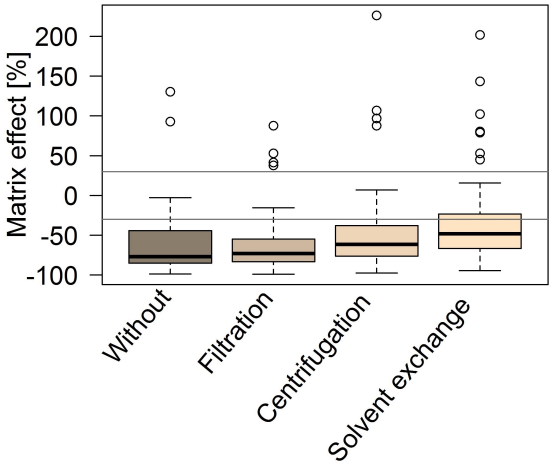

Figure C.4: Effect of different procedures prior to the RAM on the matrix effects

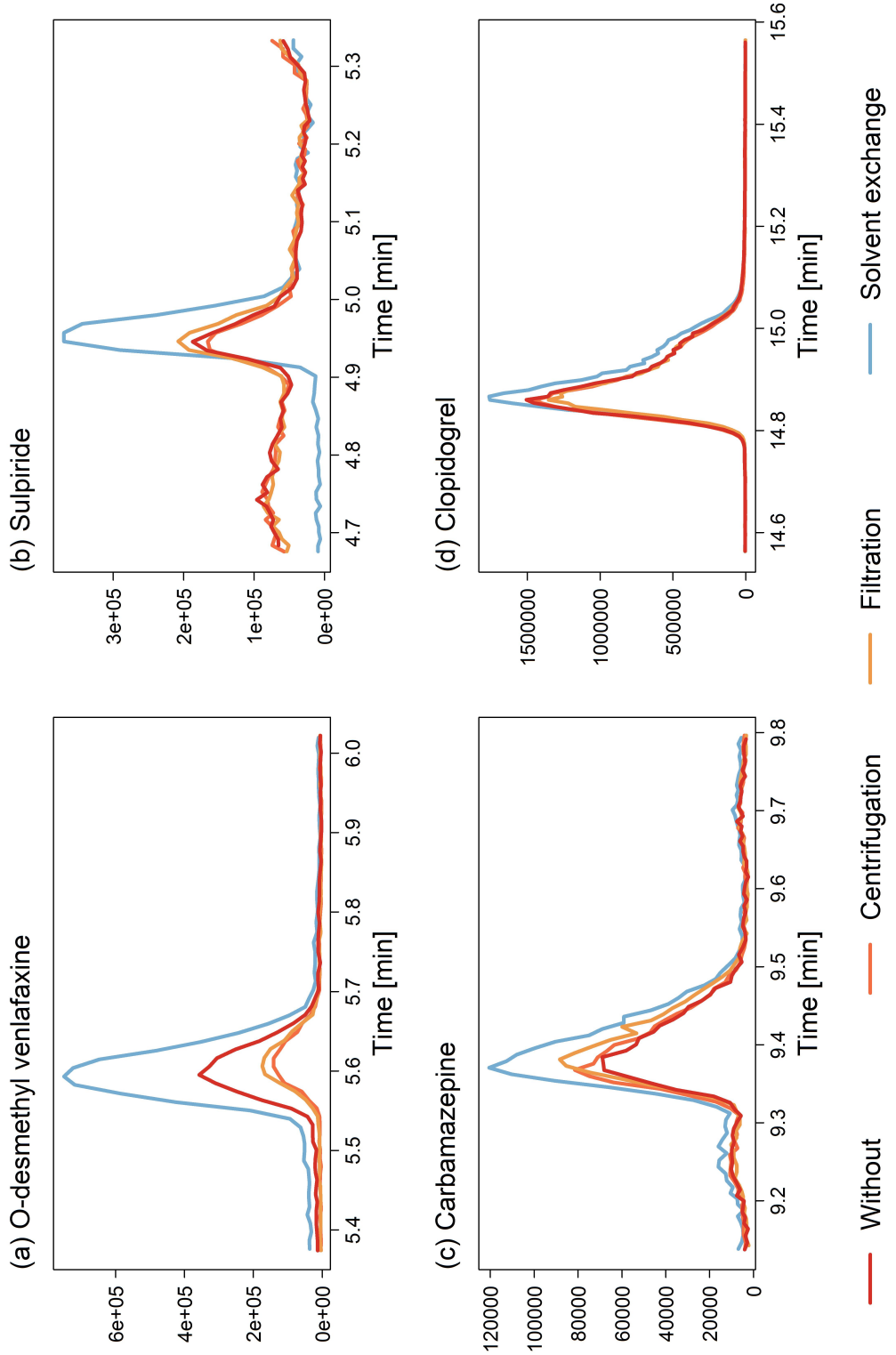

Figure C.5: Chromatogram examples showing the effects of different procedures prior to the RAM on the matrix effects

Table C.11: Relative recoveries and LOQ for the investigated analytes

	Relative recoveries (n=4) [%]									LOQ		
	Fillet			Liver			Plasma			Fillet	Liver	Plasma
Analyte	5 ng/g	10 ng/g	50 ng/g	5 ng/g	10 ng/g	50 ng/g	1 ng/mL	2.5 ng/mL	10 ng/mL	[ng/g]	[ng/g]	[ng/mL]
Amisulpride	84 ± 4	96 ± 12	97 ± 16	89 ± 6	92 ± 6	86 ± 5	84 ± 3	80 ± 3	79 ± 3	0.40	0.55	0.091
Bezafibrate	78 ± 4	82 ± 12	83 ± 14	102 ± 12	95 ± 8	89 ± 6	95 ± 4	97 ± 5	101 ± 4	0.49	0.94	0.18
Bicalutamide	84 ± 3	88 ± 9	88 ± 16	91 ± 7	94 ± 6	89 ± 1	93 ± 2	94 ± 2	93 ± 1	0.20	0.46	0.05
Candesartan	<LOQ	89 ± 14	91 ± 15	<LOQ	<LOQ	77 ± 7	<LOQ	85 ± 6	87 ± 1	5.30	39.1	1.50
Carbamazepine	91 ± 4	98 ± 10	96 ± 15	94 ± 6	96 ± 8	89 ± 2	97 ± 2	98 ± 3	98 ± 1	0.73	1.64	0.17
2-Hydroxycarbamazepine	80 ± 9	85 ± 10	83 ± 16	92 ± 9	93 ± 7	83 ± 6	105 ± 2	98 ± 3	100 ± 4	0.45	1.96	0.15
3-Hydroxycarbamazepine	79 ± 6	80 ± 6	79 ± 16	91 ± 11	89 ± 2	75 ± 6	91 ± 4	89 ± 6	93 ± 5	0.64	2.83	0.21
10,11-Dihydro-10-hydroxycarbamazepine	93 ± 7	97 ± 12	93 ± 19	<LOQ	96 ± 6	90 ± 7	94 ± 5	97 ± 5	94 ± 2	2.70	7.26	0.62
10,11-Dihydroxy-10,11-dihydrocarbamazepine	76 ± 3	79 ± 7	76 ± 13	<LOQ	<LOQ	76 ± 7	88 ± 9	85 ± 6	87 ± 4	1.40	10.6	0.23
Acridone	78 ± 5	79 ± 4	77 ± 9	88 ± 7	84 ± 7	82 ± 5	86 ± 9	86 ± 3	86 ± 5	0.62	1.34	0.11
Cetirizine	84 ± 2	87 ± 8	86 ± 16	<LOQ	<LOQ	117 ± 17	99 ± 6	128 ± 20	92 ± 2	4.50	16.5	0.44
Chlorothiazide	102 ± 4	108 ± 12	108 ± 19	109 ± 17	117 ± 4	96 ± 9	106 ± 11	109 ± 10	105 ± 9	0.51	1.14	0.12
Citalopram	84 ± 7	96 ± 6	94 ± 16	85 ± 12	90 ± 10	87 ± 2	88 ± 5	85 ± 4	85 ± 4	1.20	4.73	0.26
Desmethylcitalopram	69 ± 5	78 ± 7	75 ± 11	72 ± 9	71 ± 9	60 ± 5	81 ± 3	84 ± 5	83 ± 5	1.20	2.70	0.38
Didemethylcitalopram	87 ± 8	95 ± 10	92 ± 17	<LOQ	<LOQ	80 ± 8	97 ± 11	91 ± 10	89 ± 3	3.00	25.2	0.66
Clopidogrel	80 ± 11	87 ± 15	76 ± 15	70 ± 12	70 ± 20	62 ± 5	99 ± 2	93 ± 7	96 ± 5	0.10	0.65	0.073
Clopidogrel carboxylic acid	97 ± 4	103 ± 14	104 ± 17	<LOQ	91 ± 8	71 ± 3	105 ± 2	102 ± 1	101 ± 2	1.90	8.57	0.22
Diclofenac	106 ± 8	122 ± 34	100 ± 19	110 ± 26	102 ± 4	89 ± 5	109 ± 30	97 ± 7	93 ± 14	1.48	2.52	0.41
Diphenhydramine	83 ± 6	91 ± 8	89 ± 14	94 ± 5	95 ± 6	86 ± 6	83 ± 1	83 ± 5	83 ± 3	0.29	0.67	0.22
N-Desmethyl diphenhydramine	84 ± 2	86 ± 7	85 ± 9	90 ± 11	77 ± 10	68 ± 9	84 ± 4	84 ± 5	82.1 ± 0.5	0.91	2.60	0.21
Fexofenadine	105 ± 3	110 ± 17	106 ± 21	<LOQ	<LOQ	87 ± 4	77 ± 5	80 ± 2	78 ± 1	1.60	14.2	0.28
Flecainide	93 ± 3	98 ± 8	95 ± 15	105 ± 8	103 ± 9	99 ± 3	90 ± 2	93 ± 1	91 ± 2	0.17	0.78	0.077
Flecainide-meta-O-dealkylated	81 ± 4	84 ± 12	89 ± 17	86 ± 17	83 ± 3	96 ± 6	79 ± 4	80 ± 6	81 ± 2	0.37	1.19	0.11

	Relative recoveries (n=4) [%]									LOQ		
	Fillet			Liver			Plasma			Fillet	Liver	Plasma
Analyte	5 ng/g	10 ng/g	50 ng/g	5 ng/g	10 ng/g	50 ng/g	1 ng/mL	2.5 ng/mL	10 ng/mL	[ng/g]	[ng/g]	[ng/mL]
Fluconazole	89 ± 3	98 ± 11	98 ± 16	97 ± 8	98 ± 7	90 ± 2	99 ± 1	99 ± 2	100 ± 2	0.43	1.32	0.21
Fluoxetine	77 ± 7	76 ± 16	81 ± 14	<LOQ	97 ± 4	86 ± 10	80 ± 7	81 ± 5	86 ± 3	4.70	7.49	0.47
Norfluoxetine	78 ± 22	81 ± 17	81 ± 6	<LOQ	<LOQ	82 ± 23	79 ± 6	81 ± 8	83 ± 5	5.50	34.9	0.83
Furosemide	119 ± 4	122 ± 20	122 ± 30	<LOQ	<LOQ	74 ± 2	<LOQ	<LOQ	91 ± 7	3.20	17.8	5.50
Gabapentin lactam	87 ± 6	88 ± 4	85 ± 16	98 ± 6	95 ± 7	89 ± 3	98 ± 4	100 ± 8	99 ± 1	1.70	1.51	0.54
Hydrochlorothiazide	84 ± 7	88 ± 12	89 ± 9	104 ± 8	100 ± 20	81 ± 9	96 ± 10	86 ± 12	97 ± 6	1.30	3.31	0.33
Lamotrigine	91 ± 10	94 ± 4	88 ± 15	<LOQ	<LOQ	72 ± 4	<LOQ	86 ± 11	90 ± 7	4.80	21.7	1.20
Lidocaine	98 ± 7	100 ± 9	100 ± 14	106 ± 12	108 ± 7	89 ± 2	94 ± 3	93 ± 3	93 ± 1	1.30	1.69	0.19
Norlidocaine	133 ± 36	131 ± 15	94 ± 24	<LOQ	120 ± 10	97 ± 6	94 ± 13	94 ± 8	91 ± 6	1.80	6.74	0.90
Metoprolol	93 ± 9	98 ± 14	91 ± 15	<LOQ	110 ± 10	91 ± 7	<LOQ	96 ± 4	99 ± 11	3.00	9.34	1.30
O-Desmethyl metoprolol	<LOQ	<LOQ	114 ± 15	<LOQ	<LOQ	97 ± 3	<LOQ	103 ± 9	99 ± 5	19.0	25.3	1.80
Oxazepam	90 ± 3	94 ± 10	95 ± 16	98 ± 7	99 ± 7	82 ± 3	92 ± 3	93 ± 2	95 ± 2	1.30	3.28	0.48
Phenytoin	74 ± 10	73 ± 8	80 ± 12	97 ± 13	100 ± 20	61 ± 8	90 ± 8	81 ± 15	85 ± 13	0.73	3.74	0.18
Pregabalin lactam	<LOQ	<LOQ	73 ± 16	<LOQ	<LOQ	70 ± 4	<LOQ	<LOQ	91 ± 8	11.0	25.1	3.60
Primidone	95 ± 6	99 ± 9	100 ± 15	<LOQ	<LOQ	102 ± 3	93 ± 13	99 ± 5	99 ± 2	2.00	7.35	0.58
Quetiapine	83 ± 4	90 ± 9	91 ± 16	<LOQ	91 ± 5	70 ± 7	76 ± 13	76 ± 10	75 ± 15	0.51	7.23	0.13
Quetiapine sulfoxide	<LOQ	90 ± 12	90 ± 16	<LOQ	<LOQ	98 ± 11	<LOQ	103 ± 10	107 ± 9	7.00	25.9	1.50
Sertraline	<LOQ	66 ± 10	71 ± 12	<LOQ	80 ± 20	71 ± 16	78 ± 20	84 ± 4	81 ± 9	9.40	9.58	0.91
Sitagliptin	82 ± 2	91 ± 12	92 ± 18	<LOQ	93 ± 4	74 ± 4	83 ± 3	83 ± 3	88 ± 2	1.20	6.54	0.45
Sulfamethoxazole	87 ± 6	93 ± 15	95 ± 16	<LOQ	<LOQ	70 ± 6	<LOQ	99 ± 13	109 ± 10	4.80	16.8	1.10
N-Acetyl sulfamethoxazole	<LOQ	<LOQ	88 ± 15	<LOQ	<LOQ	115 ± 14	<LOQ	125 ± 9	134 ± 14	14.0	48.2	1.60
Sulpiride	77 ± 16	86 ± 12	81 ± 22	109 ± 9	106 ± 5	99 ± 5	91 ± 2	81 ± 3	83 ± 7	4.30	3.32	0.73
Telmisartan	98 ± 4	102 ± 13	96 ± 11	79 ± 21	80 ± 20	77 ± 10	83 ± 17	85 ± 18	84 ± 22	0.59	0.96	0.086
Torasemide	83 ± 4	89 ± 13	89 ± 16	83 ± 9	87 ± 7	76 ± 2	92 ± 2	93 ± 5	94 ± 1	0.28	0.62	0.087
Hydroxytorasemide	85 ± 7	88 ± 14	88 ± 14	85 ± 9	90 ± 7	80 ± 3	88 ± 3	88 ± 2	89 ± 6	0.35	1.78	0.28
Tramadol	92 ± 3	95 ± 10	95 ± 15	89 ± 15	94 ± 6	88 ± 4	92 ± 2	89 ± 4	89 ± 2	0.49	1.39	0.16
O-Desmethyl tramadol	<LOQ	<LOQ	100 ± 22	<LOQ	<LOQ	88 ± 3	<LOQ	<LOQ	107 ± 5	16.0	20.1	3.40

	Relative recoveries (n=4) [%]									LOQ		
	Fillet			Liver			Plasma			Fillet	Liver	Plasma
Analyte	5 ng/g	10 ng/g	50 ng/g	5 ng/g	10 ng/g	50 ng/g	1 ng/mL	2.5 ng/mL	10 ng/mL	[ng/g]	[ng/g]	[ng/mL]
N-Desmethyl tramadol	92 ± 5	96 ± 12	97 ± 18	102 ± 10	103 ± 9	97 ± 5	83 ± 16	89 ± 10	88 ± 3	0.81	2.37	0.34
N,O-Didesmethyl tramadol	<LOQ	<LOQ	100 ± 18	<LOQ	100 ± 10	91 ± 4	102 ± 11	101 ± 5	99 ± 4	11.0	9.07	0.48
Trimethoprim	91 ± 7	93 ± 8	94 ± 18	93 ± 6	98 ± 7	93 ± 6	90 ± 4	84 ± 4	88 ± 3	0.82	2.01	0.16
3-Desmethyl trimethoprim	72 ± 21	91 ± 22	71 ± 21	110 ± 23	120 ± 20	77 ± 6	88 ± 7	83 ± 7	87 ± 4	1.00	2.77	0.18
5-(3,4,5-Trimethoxybenzoyl)-2,4-pyrimidinediamine	76 ± 8	83 ± 11	82 ± 13	<LOQ	<LOQ	86 ± 4	83 ± 2	75 ± 2	80 ± 3	4.10	22.4	0.29
Valsartan	<LOQ	97 ± 14	92 ± 23	<LOQ	<LOQ	82 ± 11	<LOQ	91 ± 3	91 ± 6	5.30	36.6	1.70
Valeryl-4-hydroxyvalsartan	85 ± 18	87 ± 10	71 ± 16	<LOQ	104 ± 8	98 ± 14	<LOQ	76 ± 14	70 ± 11	2.90	8.40	1.10
Valsartanic acid	89 ± 22	106 ± 35	101 ± 15	<LOQ	<LOQ	105 ± 11	101 ± 19	101 ± 10	111 ± 14	3.20	15.8	0.59
Venlafaxine	88 ± 3	93 ± 9	94 ± 13	89 ± 9	90 ± 10	78 ± 7	88 ± 15	95 ± 23	112 ± 26	2.40	1.68	0.76
N-Desmethyl venlafaxine	88 ± 2	93 ± 9	93 ± 15	<LOQ	100 ± 20	80 ± 8	<LOQ	80 ± 1	80 ± 1	3.50	9.05	1.10
O-Desmethyl venlafaxine	116 ± 3	112 ± 8	117 ± 14	<LOQ	<LOQ	128 ± 5	<LOQ	94 ± 8	88 ± 2	5.65	12.4	1.00
N,O-Desmethyl venlafaxine	<LOQ	98 ± 15	96 ± 15	<LOQ	<LOQ	73 ± 2	129 ± 7	121 ± 11	102 ± 4	5.40	11.6	0.72
Xipamide	77 ± 4	80 ± 10	83 ± 12	85 ± 12	91 ± 8	95 ± 3	88 ± 6	88 ± 5	97 ± 7	0.15	0.52	0.081

Table C.12: Intra-day and inter-day precision

	Intra-day (n=5) [%]						Inter-day (n=4) [%]					
	Fillet		Liver		Plasma		Fillet		Liver		Plasma	
Analyte	10 ng/g	50 ng/g	10 ng/g	50 ng/g	2.5 ng/mL	10 ng/mL	10 ng/g	50 ng/g	10 ng/g	50 ng/g	2.5 ng/mL	10 ng/mL
Amisulpride	1.4	1.7	1.8	2.3	1.9	1.9	2.3	2.2	1.0	2.4	1.9	1.6
Bezafibrate	2.0	1.3	2.2	1.8	1.4	2.3	1.3	1.0	2.1	1.9	1.9	2.5
Bicalutamide	0.9	0.1	1.2	1.2	0.9	1.7	1.7	2.4	0.6	1.9	2.2	0.3
Candesartan	2.5	2.5	<LOQ	8.1	1.7	1.2	3.5	2.7	<LOQ	5.2	1.2	3.7
Carbamazepine	1.9	0.3	1.8	1.7	0.6	0.8	1.1	1.5	1.7	1.6	1.8	0.7
2-Hydroxycarbamazepine	2.4	1.9	1.5	0.7	1.7	1.5	2.4	0.8	5.0	5.3	2.7	3.2
3-Hydroxycarbamazepine	2.4	1.1	2.1	0.9	3.4	2.2	0.6	1.6	5.6	2.6	3.9	1.8
10,11-Dihydro-10-hydroxycarbamazepine	1.3	1.2	2.9	2.9	2.3	1.1	2.7	3.4	2.7	4.2	3.3	1.5
10,11-Dihydroxy-10,11-dihydrocarbamazepine	3.5	1.5	<LOQ	2.4	2.7	1.5	3.9	2.6	<LOQ	0.9	4.9	5.8
Acridone	2.0	0.8	2.1	1.0	0.8	2.9	1.7	0.8	0.9	0.9	4.3	4.8
Cetirizine	2.2	2.0	<LOQ	3.4	2.3	1.6	7.9	7.9	<LOQ	0.6	2.4	6.1
Chlorothiazide	4.1	1.5	3.6	3.7	5.2	3.5	4.5	4.9	6.0	5.6	2.9	6.4
Citalopram	3.2	2.7	2.3	1.2	1.9	2.2	2.3	2.2	2.3	3.7	1.9	2.4
Desmethylcitalopram	1.3	3.1	5.1	1.5	1.4	2.3	3.5	3.0	10.3	4.1	6.9	8.4
Didemethylcitalopram	1.8	2.6	<LOQ	5.8	5.2	1.9	2.7	1.6	<LOQ	7	3.3	2.5
Clopidogrel	0.4	1.1	1.2	1.0	0.6	0.4	2.1	1.7	0.8	4.8	0.6	0.3
Clopidogrel carboxylic acid	1.6	1.1	2.1	2.0	1.3	0.7	2.4	1.5	2.8	4.7	1.9	0.5
Diclofenac	2.2	2.4	4.5	7.1	1.9	4.8	3.4	0.8	4.2	5.4	2.9	0.7
Diphenhydramine	1.7	1.2	2.4	1.7	2.2	2.9	1.1	0.9	3.6	2.1	2.7	2.3
N-Desmethyl diphenhydramine	2.8	1.4	4.7	4.6	1.9	2.1	0.5	2.8	9.6	7.7	3.5	2.4
Fexofenadine	2.2	2.6	<LOQ	3.8	2.3	1.5	3.7	3.9	<LOQ	3.7	3.4	1.4
Flecainide	1.9	3.2	3.2	2.4	1.9	1.4	3.8	1.0	2.8	1.4	0.7	1.9
Flecainide-meta-O-dealkylated	1.9	2.0	2.8	3.4	1.9	2.6	6.0	5.8	5.8	13.8	1.9	3.0
Fluconazole	0.6	0.4	1.2	0.8	1.7	1.3	2.5	0.9	3.6	1.9	3.1	1.1

	Intra-day (n=5) [%]						Inter-day (n=4) [%]					
	Fillet		Liver		Plasma		Fillet		Liver		Plasma	
Analyte	10 ng/g	50 ng/g	10 ng/g	50 ng/g	2.5 ng/mL	10 ng/mL	10 ng/g	50 ng/g	10 ng/g	50 ng/g	2.5 ng/mL	10 ng/mL
Fluoxetine	4.5	4.6	10.6	6.8	2.8	2.3	3.9	0.9	1.9	2.8	3.7	6.7
Norfluoxetine	10.3	12.8	<LOQ	12.9	5.2	3.4	6.6	10.9	<LOQ	12.9	8.4	2.6
Furosemide	1.5	2.0	<LOQ	2.9	<LOQ	4.1	1.4	4.9	<LOQ	11.1	<LOQ	9.3
Gabapentin lactam	2.1	3.5	2.5	3.4	5.6	3.2	2.1	2.3	2.5	2.5	3.4	3.9
Hydrochlorothiazide	6.0	5.3	5.9	6.8	8.4	5.9	8.0	2.1	6.0	7.3	8.7	5.3
Lamotrigine	2.6	4.9	<LOQ	2.4	7.7	4.2	7.4	3.6	<LOQ	2.8	9.8	4.4
Lidocaine	1.8	1.0	1.6	0.8	1.2	2.1	1.3	2.2	0.6	2.3	2.6	1.6
Norlidocaine	2.0	7.5	5.0	0.8	1.4	4.0	4.9	6.8	7.2	8.1	5.0	4.1
Metoprolol	2.0	2.6	1.3	2.5	5.3	4.4	5.6	1.1	5.4	3.6	1.7	2.0
O-Desmethyl metoprolol	<LOQ	3.9	<LOQ	3.4	5.1	2.7	<LOQ	7.1	<LOQ	3.5	7.6	2.3
Oxazepam	1.5	1.2	3.6	1.8	1.3	1.0	1.3	1.2	3.1	12.1	2.9	1.5
Phenytoin	3.4	2.8	3.4	1.8	3.2	2.8	4.2	1.1	0.9	8.9	12	15.6
Pregabalin lactam	<LOQ	3.2	<LOQ	2.3	<LOQ	3.4	<LOQ	4.1	<LOQ	4.5	<LOQ	5.1
Primidone	1.8	1.4	<LOQ	2.8	2.1	1.0	2.0	2.3	<LOQ	3.1	4.6	1.1
Quetiapine	1.8	0.8	4.1	1.4	1.0	2.7	3.0	4.3	9.4	4.4	7.6	4.4
Quetiapine sulfoxide	1.3	1.1	<LOQ	1.0	1.8	1.8	4.3	4.9	<LOQ	10.1	7.2	2.2
Sertraline	10.5	15	14.2	8.9	4.1	8.3	44.5	18.5	34.6	5.1	4.5	11
Sitagliptin	1.2	0.9	1.9	1.8	1.2	1.3	2.4	2.6	2.9	2.3	2.1	0.8
Sulfamethoxazole	6.1	7.2	<LOQ	5.8	6.7	4.4	5.3	2.3	<LOQ	3.1	5.9	6.7
N-Acetyl sulfamethoxazole	<LOQ	2.8	<LOQ	2.8	4.3	2.8	<LOQ	3.1	<LOQ	3.6	7.9	10.3
Sulpiride	5.8	4.8	1.8	2.8	3.7	1.2	2.7	2.1	2.9	2.1	4.5	4.3
Telmisartan	1.8	1.9	4.7	7.7	8.2	5.5	1.7	1.0	10.3	7.1	7.4	18.1
Torasemide	2.1	2.1	1.0	1.2	2.2	0.9	1.2	1.3	1.0	2.5	1.8	1.9
Hydroxytorasemide	2.2	4.4	5.7	3.0	2.5	1.9	2.9	1.8	2.3	3.5	2.3	4.6
Tramadol	0.8	1.3	1.1	1.3	1.8	0.9	0.5	0.7	1.4	2.0	1.2	0.3
O-Desmethyl tramadol	<LOQ	0.4	<LOQ	3.2	<LOQ	2.6	<LOQ	3.0	<LOQ	1.3	<LOQ	3.5
N-Desmethyl tramadol	1.0	0.3	3.1	2.1	1.0	2.0	2.2	1.2	2.5	2.8	7.1	2.7

	Intra-day (n=5) [%]						Inter-day (n=4) [%]					
	Fillet		Liver		Plasma		Fillet		Liver		Plasma	
Analyte	10 ng/g	50 ng/g	10 ng/g	50 ng/g	2.5 ng/mL	10 ng/mL	10 ng/g	50 ng/g	10 ng/g	50 ng/g	2.5 ng/mL	10 ng/mL
N,O-Didesmethyl tramadol	<LOQ	2.2	7.5	3.1	5.5	1.6	<LOQ	4.2	7.3	2.6	1.8	2.2
Trimethoprim	3.1	2.4	2.1	3.6	2.7	1.2	1.8	3.5	0.9	1.1	3.5	14.8
3-Desmethyl trimethoprim	0.4	1.4	3.3	2.7	1.0	1.4	14.2	10.5	17.3	6.0	6.9	6.5
5-(3,4,5-Trimethoxybenzoyl)-2,4-pyrimidinediamine	1.1	1.0	<LOQ	1.8	1.5	1.3	2.5	2.2	<LOQ	5.5	2.8	1.8
Valsartan	3.7	4.5	<LOQ	12.2	3.4	2.9	6.4	3.3	<LOQ	1.9	15	6.8
Valeryl-4-hydroxyvalsartan	4.1	3.2	9.0	5.2	6.8	3.2	5.5	3.6	9.6	4.9	7.9	4.5
Valsartanic acid	4.5	4.2	<LOQ	14.6	4.2	7.2	6.4	2.9	<LOQ	12.3	10.5	8.8
Venlafaxine	1.5	1.0	1.0	1.2	5.0	4.3	2.3	2.3	1.7	1.8	10.1	3.3
N-Desmethyl venlafaxine	1.8	1.1	4.6	1.0	1.7	0.7	1.2	1.2	9.0	1.1	2.0	1.7
O-Desmethyl venlafaxine	2.2	0.8	<LOQ	3.7	3.6	0.5	7.3	5.2	<LOQ	16.2	3.0	3.4
N,O-Desmethyl venlafaxine	2.3	0.9	<LOQ	3.3	3.3	1.3	1.7	1.3	<LOQ	6.7	3.6	1.9
Xipamide	3.2	1.2	1.3	2.1	3.9	4.4	4.5	5.1	5.3	6.0	6.4	5.2

Table C.13: Matrix effects with their 95% confidence interval

	Matrix effects (n=4) [%]		
	Fillet	Liver	Plasma
Amisulpride	90 ± 20	140 ± 20	-23 ± 9
Bezafibrate	-28 ± 6	-52 ± 5	-28 ± 7
Bicalutamide	-23 ± 2	-24 ± 6	-35 ± 4
Candesartan	-14 ± 6	-56 ± 6	-10 ± 10
Carbamazepine	-31 ± 5	-40 ± 5	-31 ± 8
2-Hydroxycarbamazepine	-51 ± 3	-55 ± 5	-39 ± 8
3-Hydroxycarbamazepine	-46 ± 4	-54 ± 4	-49 ± 5
10,11-Dihydro-10-hydroxycarbamazepine	-46 ± 5	-54 ± 5	-46 ± 6
10,11-Dihydroxy-10,11-dihydrocarbamazepine	-49 ± 5	-51 ± 5	-51 ± 6
Acridone	-46 ± 2	-51 ± 5	-43 ± 5
Cetirizine	-48 ± 2	-42 ± 5	-41.3 ± 0.8
Chlorothiazide	-52 ± 6	-37 ± 8	-49 ± 3
Citalopram	15 ± 3	36 ± 9	-26 ± 4
Desmethylcitalopram	8 ± 7	20 ± 10	-28 ± 6
Didemethylcitalopram	9 ± 8	10 ± 8	-14 ± 8
Clopidogrel	-6 ± 4	-19 ± 6	4 ± 4
Clopidogrel carboxylic acid	-20 ± 5	-24 ± 7	-9 ± 7
Diclofenac	-43 ± 7	-79 ± 5	-50 ± 20
Diphenhydramine	5 ± 4	3 ± 9	-32 ± 5
N-Desmethyl diphenhydramine	-11 ± 5	-10 ± 10	-43 ± 5
Fexofenadine	13 ± 6	2 ± 8	-18 ± 6
Flecainide	8 ± 5	30 ± 10	-20 ± 6
Flecainide-meta-O-dealkylated	-14 ± 5	-2 ± 6	-31 ± 7
Fluconazole	-26 ± 5	-27 ± 7	-22 ± 5
Fluoxetine	80 ± 10	90 ± 30	30 ± 10
Norfluoxetine	49 ± 7	40 ± 20	30 ± 10
Furosemide	-22 ± 5	-60 ± 10	-33 ± 9
Gabapentin lactam	-38 ± 6	-39 ± 5	-35 ± 6
Hydrochlorothiazide	-84 ± 2	-74 ± 3	-88 ± 1
Lamotrigine	-39 ± 4	-38 ± 5	-63 ± 4
Lidocaine	-30 ± 5	-31 ± 6	-58 ± 4
Norlidocaine	-30 ± 6	-36 ± 5	-58 ± 3
Metoprolol	-23 ± 5	2 ± 8	-64 ± 4
O-Desmethyl metoprolol	-31 ± 9	-20 ± 8	-59 ± 3
Oxazepam	-32 ± 4	-67 ± 3	-19 ± 5
Phenytoin	-82 ± 2	-86 ± 3	-56 ± 9
Pregabalin lactam	-27 ± 10	-31 ± 10	-27 ± 8
Primidone	-75 ± 4	-74 ± 4	-81 ± 3
Quetiapine	115 ± 10	140 ± 20	-3 ± 7
Quetiapine sulfoxide	-42 ± 4	-35 ± 5	-36 ± 9
Sertraline	45 ± 9	0 ± 20	-20 ± 10
Sitagliptin	-25 ± 4	-20 ± 5	-48 ± 5
Sulfamethoxazole	-62 ± 3	-80 ± 4	-59 ± 8
N-Acetyl sulfamethoxazole	-78 ± 1	-69 ± 5	-50 ± 10
Sulpiride	9 ± 17	40 ± 10	-48 ± 4
Telmisartan	146 ± 31	200 ± 40	130 ± 20

	Matrix effects(n=4) [%]		
	Fillet	Liver	Plasma
Torasemide	-3 ± 5	-1 ± 7	6 ± 2
Hydroxytorasemide	-20 ± 5	-17 ± 7	-47 ± 4
Tramadol	-26 ± 5	-20 ± 10	-65 ± 4
O-Desmethyl tramadol	-38 ± 9	-45 ± 8	-67 ± 5
N-Desmethyl tramadol	-32 ± 4	-24 ± 7	-63 ± 5
N,O-Didesmethyl tramadol	-37 ± 6	-37 ± 8	-64 ± 3
Trimethoprim	-22 ± 8	-15 ± 5	-50 ± 8
3-Desmethyl trimethoprim	-29 ± 9	-24 ± 7	-59 ± 4
5-(3,4,5-Trimethoxybenzoyl)-2,4-pyrimidinediamine	-40 ± 7	-31 ± 8	-41 ± 7
Valsartan	-1 ± 7	-55 ± 6	-30 ± 10
Valeryl-4-hydroxyvalsartan	8 ± 6	3 ± 10	-35 ± 4
Valsartanic acid	-71 ± 4	-76 ± 3	-50 ± 10
Venlafaxine	-11 ± 6	-7 ± 8	-45 ± 5
N-Desmethyl venlafaxine	-20 ± 6	-19 ± 10	-56 ± 4
O-Desmethyl venlafaxine	-36 ± 5	-25 ± 9	-61 ± 2
N,O-Desmethyl venlafaxine	-36 ± 4	-31 ± 8	-59 ± 6
Xipamide	2 ± 3	0 ± 10	-20 ± 9

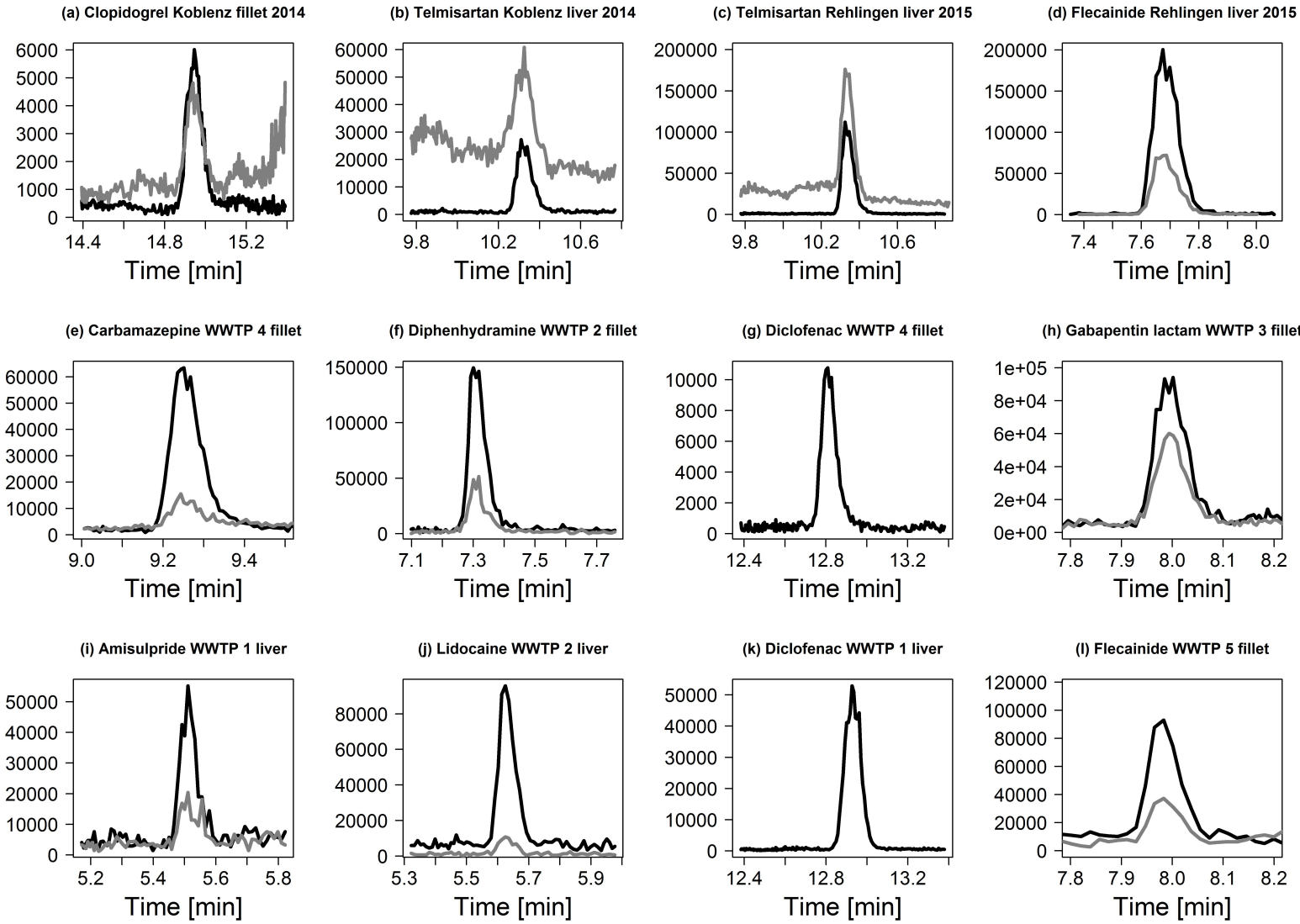

Figure C.6: Example of XIC for the detected analytes in the studied environmental samples. The MRM of quantification is in black, whereas the MRM of confirmation is in gray.

Table C.14: Mean concentrations determined over 3 replicates for the carps kept in fish monitoring ponds fed by WWTP effluent with their 95% confidence interval

	Concentration [ng/g]									
	Pond fed by WWTP effluent 1 (n=3)		Pond fed by WWTP effluent 2 (n=3)		Pond fed by WWTP effluent 3 (n=3)		Pond fed by WWTP effluent 4 (n=3)		Pond fed by WWTP effluent 5 (n=3)	
Analyte	Fillet	Liver	Fillet	Liver	Fillet	Liver	Fillet	Liver	Fillet	Liver
Amisulpride	<LOQ	0.6 ± 0.1	<LOQ	3.1 ± 0.6	<LOD	<LOQ	0.52 ± 0.03	7.0 ± 0.9	<LOQ	2.1 ± 0.2
Bezafibrate	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Bicalutamide	<LOQ	1.6 ± 0.2	<LOQ	<LOQ	0.32 ± 0.04	2.7 ± 0.2	<LOQ	1.2 ± 0.1	<LOQ	<LOQ
Candesartan	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Carbamazepine	<LOQ	<LOQ	<LOQ	<LOQ	0.73 ± 0.08	1.7 ± 0.2	0.86 ± 0.06	2.3 ± 0.3	<LOQ	<LOQ
2-Hydroxycarbamazepine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
3-Hydroxycarbamazepine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
10,11-Dihydro-10-hydroxycarbamazepine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	4.4 ± 0.6	<LOQ	<LOQ	<LOQ
10,11-Dihydroxy-10,11-dihydrocarbamazepine	2.2 ± 0.2	<LOQ	<LOQ	<LOQ	2.5 ± 0.4	<LOQ	6 ± 2	11 ± 4	3.5 ± 0.3	<LOQ
Acridone	<LOQ	5.1 ± 0.3	2.10 ± 0.09	7.7 ± 0.3	1.33 ± 0.06	9.5 ± 0.5	0.9 ± 0.3	12 ± 1	2.1 ± 0.3	3.7 ± 0.5
Cetirizine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Chlorothiazide	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	2 ± 1	<LOQ	<LOQ
Citalopram	7 ± 1	22.1 ± 0.8	<LOQ	7 ± 1	2.8 ± 0.3	9 ± 2	3.4 ± 0.8	25 ± 4	<LOQ	<LOQ
Desmethylcitalopram	7 ± 2	30 ± 4	1.1 ± 0.5	<LOQ	3.2 ± 0.6	<LOQ	4.0 ± 0.5	32 ± 3	1.5 ± 0.4	<LOQ
Didemethylcitalopram	3.5 ± 0.6	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Clopidogrel	1.0 ± 0.1	<LOQ	0.74 ± 0.06	<LOQ	0.9 ± 0.3	<LOQ	1.0 ± 0.1	<LOQ	5.6 ± 0.2	1.1 ± 0.1
Clopidogrel carboxylic acid	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Diclofenac	12 ± 2	47 ± 7	2.6 ± 0.7	11 ± 3	9.6 ± 0.2	39 ± 4	7.6 ± 0.1	32 ± 2	16 ± 1	37 ± 3
Diphenhydramine	3.9 ± 0.7	5.8 ± 0.4	0.8 ± 0.2	4.8 ± 0.1	<LOQ	<LOQ	1.7 ± 0.3	8 ± 3	1.03 ± 0.08	2.3 ± 0.3
N-Desmethyl diphenhydramine	6.3 ± 0.8	5.4 ± 0.3	<LOQ	<LOQ	<LOQ	<LOQ	2.3 ± 0.7	6.3 ± 0.9	1.34 ± 0.07	<LOQ
Fexofenadine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Flecainide	3.4 ± 0.3	18 ± 1	0.8 ± 0.1	19 ± 7	1.32 ± 0.08	7 ± 2	1.27 ± 0.07	13 ± 2	0.29 ± 0.06	1.3 ± 0.6
Flecainide-meta-O-dealkylated	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

	Concentration [ng/g]									
	Pond fed by WWTP effluent 1 (n=3)		Pond fed by WWTP effluent 2 (n=3)		Pond fed by WWTP effluent 3 (n=3)		Pond fed by WWTP effluent 4 (n=3)		Pond fed by WWTP effluent 5 (n=3)	
Analyte	Fillet	Liver	Fillet	Liver	Fillet	Liver	Fillet	Liver	Fillet	Liver
Fluconazole	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Fluoxetine	9 ± 5	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	10 ± 3	25.8 ± 0.8	<LOQ	<LOQ
Norfluoxetine	5 ± 2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	11.0 ± 0.8	<LOQ	<LOQ	<LOQ
Furosemide	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Gabapentin lactam	4.6 ± 0.5	14 ± 1	3.0 ± 0.4	14 ± 1	4.5 ± 0.8	26 ± 2	8.1 ± 0.4	22 ± 1	<LOQ	2.8 ± 0.6
Hydrochlorothiazide	<LOQ	9 ± 1	<LOQ	<LOQ	1.6 ± 0.3	11 ± 2	1.8 ± 0.6	18 ± 8	2 ± 1	10 ± 1
Lamotrigine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Lidocaine	3.9 ± 0.1	3.4 ± 0.3	<LOQ	<LOQ	<LOQ	<LOQ	1.78 ± 0.04	<LOQ	<LOQ	<LOQ
Norlidocaine	9.6 ± 0.5	10 ± 3	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Metoprolol	<LOQ	<LOQ	<LOQ	8.0 ± 0.1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
O-Desmethyl metoprolol	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Oxazepam	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Phenytoin	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Pregabalin lactam	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Primidone	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Quetiapine	3 ± 2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Quetiapine sulfoxide	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Sertraline	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	12 ± 2	<LOQ	<LOQ
Sitagliptin	1.8 ± 0.4	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Sulfamethoxazole	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
N-Acetyl sulfamethoxazole	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Sulpiride	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Telmisartan	<LOQ	5 ± 1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	2.3 ± 0.6	<LOQ	<LOQ
Torasemide	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Hydroxytorasemide	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Tramadol	8.6 ± 0.7	15 ± 2	2.01 ± 0.09	6.2 ± 0.9	3.5 ± 0.3	2.34 ± 0.09	8 ± 3	22 ± 3	1.3 ± 0.2	3 ± 2

	Concentration [ng/g]									
	Pond fed by WWTP effluent 1 (n=3)		Pond fed by WWTP effluent 2 (n=3)		Pond fed by WWTP effluent 3 (n=3)		Pond fed by WWTP effluent 4 (n=3)		Pond fed by WWTP effluent 5 (n=3)	
Analyte	Fillet	Liver	Fillet	Liver	Fillet	Liver	Fillet	Liver	Fillet	Liver
O-Desmethyl tramadol	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	27 ± 4	<LOQ	<LOQ
N-Desmethyl tramadol	3.5 ± 0.5	4.9 ± 0.6	1.01 ± 0.06	<LOQ	1.4 ± 0.1	<LOQ	4.7 ± 0.1	13 ± 2	2.72 ± 0.07	3.3 ± 0.3
N,O-Didesmethyl tramadol	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	12.6 ± 0.3	<LOQ	<LOQ
Trimethoprim	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
3-Desmethyl trimethoprim	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
5-(3,4,5-Trimethoxybenzoyl)-2,4-pyrimidinediamine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Valsartan	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Valeryl-4-hydroxyvalsartan	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Valsartanic acid	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Venlafaxine	2.9 ± 0.3	8 ± 6	<LOQ	2.6 ± 0.3	<LOQ	3.4 ± 0.9	<LOQ	10 ± 2	<LOQ	<LOQ
N-Desmethyl venlafaxine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
O-Desmethyl venlafaxine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	24 ± 1	<LOQ	<LOQ
N,O-Desmethyl venlafaxine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Xipamide	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

Table C.15: Mean concentrations determined over 3 replicates for the investigated Teltow Canal breams with their 95% confidence interval (first part)

	Concentration [ng/g]											
	U1 (n=3)		U2 (n=3)		U3 (n=3)		U4 (n=3)		U5 (n=3)		U6 (n=3)	
Analyte	Fillet	Liver	Fillet	Liver	Fillet	Liver	Fillet	Liver	Fillet	Liver	Fillet	Liver
Amisulpride	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Bezafibrate	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Bicalutamide	<LOQ	0.66 ± 0.04	<LOQ	0.47 ± 0.04	<LOQ	0.55 ± 0.05	<LOQ	0.79 ± 0.02	<LOQ	0.59 ± 0.04	<LOQ	0.59 ± 0.09
Candesartan	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Carbamazepine	0.99 ± 0.01	2.10 ± 0.09	1.23 ± 0.05	2.18 ± 0.19	0.92 ± 0.04	1.69 ± 0.09	1.01 ± 0.06	2.13 ± 0.07	1.01 ± 0.01	1.76 ± 0.06	0.73 ± 0.04	<LOQ
2-Hydroxycarbamazepine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
3-Hydroxycarbamazepine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
10,11-Dihydro-10-hydroxy-carbamazepine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
10,11-Dihydroxy-10,11-dihydrocarbamazepine	1.49 ± 0.02	<LOQ	2.0 ± 0.1	<LOQ	1.6 ± 0.1	<LOQ	1.7 ± 0.2	<LOQ	1.83 ± 0.04	<LOQ	1.76 ± 0.07	<LOQ
Acridone	<LOQ	<LOQ	<LOQ	1.6 ± 0.2	<LOQ	2.8 ± 0.3	<LOQ	2.3 ± 0.4	<LOQ	2.56 ± 0.09	<LOQ	2.0 ± 0.5
Cetirizine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Chlorothiazide	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Citalopram	<LOQ	8.6 ± 0.9	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Desmethylcitalopram	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.17 ± 0.08	<LOQ	1.3 ± 0.08	<LOQ	<LOQ	<LOQ
Didemethylcitalopram	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Clopidogrel	1.61 ± 0.06	1.9 ± 0.4	2.8 ± 0.2	1.2 ± 0.4	1.8 ± 0.3	1.3 ± 0.6	1.69 ± 0.08	<LOQ	3.3 ± 0.4	0.9 ± 0.5	1.79 ± 0.10	2.2 ± 0.4
Clopidogrel carboxylic acid	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Diclofenac	6.3 ± 0.2	11.5 ± 0.9	6.2 ± 0.8	10.3 ± 0.1	3 ± 1	4.83 ± 0.09	3.1 ± 0.4	4.9 ± 0.3	3.8 ± 0.8	5.5 ± 0.5	4.3 ± 0.5	9 ± 2
Diphenhydramine	0.76 ± 0.05	5.3 ± 0.2	1.12 ± 0.08	7.8 ± 0.5	0.88 ± 0.03	4.6 ± 0.4	0.77 ± 0.02	4.0 ± 0.6	0.9 ± 0.1	4.4 ± 0.3	0.64 ± 0.03	4.0 ± 0.6
N-Desmethyl diphenhydramine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Fexofenadine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Flecainide	0.430 ± 0.005	2.2 ± 0.1	0.50 ± 0.02	2.6 ± 0.6	0.42 ± 0.02	1.59 ± 0.09	0.48 ± 0.07	2.0 ± 0.2	0.47 ± 0.02	2.0 ± 0.1	0.32 ± 0.07	2.1 ± 0.4
Flecainide-meta-O-dealkylated	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Fluconazole	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Fluoxetine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Norfluoxetine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Furosemide	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Gabapentin lactam	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	2.2 ± 0.2	<LOQ	2.0 ± 0.4	<LOQ	<LOQ
Hydrochlorothiazide	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.4 ± 0.2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Lamotrigine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Lidocaine	1.51 ± 0.08	<LOQ	2.5 ± 0.1	3.6 ± 0.2	1.99 ± 0.08	2.1 ± 0.2	1.7 ± 0.2	<LOQ	1.6 ± 0.3	2.08 ± 0.06	2.0 ± 0.1	4.3 ± 0.1
Norlidocaine	12 ± 2	20 ± 2	8.8 ± 0.8	15.3 ± 0.9	8.8 ± 0.8	<LOQ	12 ± 1	17 ± 2	11 ± 1	16.4 ± 0.8	5 ± 1	8.8 ± 0.9
Metoprolol	<LOQ	<LOQ	3.2 ± 0.3	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	3.8 ± 0.4	11 ± 1
O-Desmethyl metoprolol	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

	Concentration [ng/g]											
	U1 (n=3)		U2 (n=3)		U3 (n=3)		U4 (n=3)		U5 (n=3)		U6 (n=3)	
Analyte	Fillet	Liver	Fillet	Liver	Fillet	Liver	Fillet	Liver	Fillet	Liver	Fillet	Liver
Oxazepam	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Phenytoin	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Pregabalin lactam	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Primidone	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Quetiapine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Quetiapine sulfoxide	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Sertraline	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Sitagliptin	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Sulfamethoxazole	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
N-Acetyl sulfamethoxazole	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Sulpiride	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Telmisartan	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Torasemide	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Hydroxytorasemide	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Tramadol	1.6 ± 0.2	2.5 ± 0.3	2.3 ± 0.2	4.5 ± 0.3	2.1 ± 0.2	2.1 ± 0.3	2.0 ± 0.2	2.1 ± 0.5	2.0 ± 0.2	2.4 ± 0.6	2.1 ± 0.3	3.8 ± 0.5
O-Desmethyl tramadol	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
N-Desmethyl tramadol	1.3 ± 0.1	3.4 ± 0.3	2.0 ± 0.2	4.6 ± 0.8	1.24 ± 0.08	<LOQ	1.39 ± 0.08	2.7 ± 0.4	1.33 ± 0.02	2 ± 1	1.7 ± 0.3	4.8 ± 0.6
N,O-Didesmethyl tramadol	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Trimethoprim	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
3-Desmethyl trimethoprim	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
5-(3,4,5-Trimethoxybenzoyl)-2,4-pyrimidinediamine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Valsartan	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Valeryl-4-hydroxyvalsartan	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Valsartanic acid	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Venlafaxine	<LOQ	<LOQ	<LOQ	3.46 ± 0.07	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
N-Desmethyl venlafaxine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
O-Desmethyl venlafaxine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	10 ± 1
N,O-Desmethyl venlafaxine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Xipamide	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

Table C.16: Mean concentrations determined over 3 replicates for the investigated Teltow Canal fish with their 95% confidence interval (second part)

	Concentration [ng/g]									
	U7 (n=3)		U8 (n=3)		U9 (n=3)		U10 (n=3)		U11 (n=3)	
Analyte	Fillet	Liver	Fillet	Liver	Fillet	Liver	Fillet	Liver	Fillet	Liver
Amisulpride	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Bezafibrate	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Bicalutamide	<LOQ	0.58 ± 0.06	<LOQ	0.61 ± 0.06	<LOQ	0.55 ± 0.01	<LOQ	0.66 ± 0.03	<LOQ	0.80 ± 0.04
Candesartan	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Carbamazepine	0.95 ± 0.04	1.8 ± 0.2	1.01 ± 0.05	1.77 ± 0.08	0.84 ± 0.05	1.6 ± 0.1	1.42 ± 0.05	2.6 ± 0.1	1.01 ± 0.03	2.2 ± 0.1
2-Hydroxycarbamazepine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
3-Hydroxycarbamazepine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
10,11-Dihydro-10-hydroxy-carbamazepine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
10,11-Dihydroxy-10,11-dihydrocarbamazepine	1.5 ± 0.1	<LOQ	1.9 ± 0.1	<LOQ	<LOQ	<LOQ	1.7 ± 0.1	<LOQ	1.6 ± 0.1	<LOQ
Acridone	<LOQ	1.9 ± 0.1	<LOQ	2.5 ± 0.6	0.72 ± 0.07	2.9 ± 0.3	<LOQ	1.5 ± 0.3	<LOQ	2.2 ± 0.2
Cetirizine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Chlorothiazide	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Citalopram	<LOQ	<LOQ	<LOQ	5.7 ± 0.9	<LOQ	<LOQ	<LOQ	6.6 ± 0.4	<LOQ	<LOQ
Desmethylcitalopram	<LOQ	<LOQ	1.48 ± 0.05	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Didemethylcitalopram	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Clopidogrel	2.0 ± 0.1	1.4 ± 0.8	2.2 ± 0.2	0.8 ± 0.4	1.52 ± 0.09	<LOQ	3.3 ± 0.3	0.8 ± 0.5	1.8 ± 0.2	0.8 ± 0.5
Clopidogrel carboxylic acid	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Diclofenac	3.1 ± 0.1	7 ± 4	2.6 ± 0.1	4.6 ± 0.7	4.8 ± 0.6	6.0 ± 0.8	3.43 ± 0.08	6 ± 4	4 ± 1	9.3 ± 0.2
Diphenhydramine	1.05 ± 0.07	7.7 ± 0.4	1.21 ± 0.06	5.7 ± 0.5	1.37 ± 0.07	6.9 ± 0.9	0.93 ± 0.08	6.7 ± 0.1	0.95 ± 0.05	5.9 ± 0.4
N-Desmethyl diphenhydramine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Fexofenadine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Flecainide	0.46 ± 0.07	2.5 ± 0.2	0.58 ± 0.08	1.8 ± 0.3	0.33 ± 0.03	1.4 ± 0.3	0.36 ± 0.03	1.8 ± 0.1	0.40 ± 0.04	1.8 ± 0.3
Flecainide-meta-O-dealkylated	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

	Concentration [ng/g]									
	U7 (n=3)		U8 (n=3)		U9 (n=3)		U10 (n=3)		U11 (n=3)	
Analyte	Fillet	Liver	Fillet	Liver	Fillet	Liver	Fillet	Liver	Fillet	Liver
Fluconazole	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Fluoxetine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Norfluoxetine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Furosemide	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Gabapentin lactam	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	2.8 ± 0.3	<LOQ	<LOQ
Hydrochlorothiazide	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Lamotrigine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Lidocaine	2.0 ± 0.2	3.5 ± 0.2	1.87 ± 0.04	2.0 ± 0.2	2.57 ± 0.09	3.7 ± 0.4	1.78 ± 0.1	2.5 ± 0.2	1.53 ± 0.06	1.9 ± 0.7
Norlidocaine	7.6 ± 0.8	17.2 ± 0.5	11.9 ± 0.2	17 ± 0.4	5.5 ± 0.5	8.0 ± 0.4	11 ± 2	22 ± 2	9.7 ± 0.7	18 ± 1
Metoprolol	4.4 ± 0.4	10 ± 2	3.7 ± 0.3	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
O-Desmethyl metoprolol	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Oxazepam	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Phenytoin	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Pregabalin lactam	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Primidone	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Quetiapine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Quetiapine sulfoxide	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Sertraline	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Sitagliptin	<LOQ	<LOQ	1.51 ± 0.04	11.6 ± 0.4	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Sulfamethoxazole	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
N-Acetyl sulfamethoxazole	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Sulpiride	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Telmisartan	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Torasemide	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Hydroxytorasemide	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Tramadol	2.1 ± 0.4	4 ± 2	2.2 ± 0.1	2.7 ± 0.4	2.1 ± 0.4	3.4 ± 0.5	1.72 ± 0.08	3.0 ± 0.2	1.78 ± 0.06	4 ± 2
O-Desmethyl tramadol	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

	Concentration [ng/g]									
	U7 (n=3)		U8 (n=3)		U9 (n=3)		U10 (n=3)		U11 (n=3)	
Analyte	Fillet	Liver	Fillet	Liver	Fillet	Liver	Fillet	Liver	Fillet	Liver
N-Desmethyl tramadol	1.6 ± 0.1	4.0 ± 0.4	1.9 ± 0.1	<LOQ	2.2 ± 0.4	<LOQ	1.7 ± 0.2	<LOQ	1.5 ± 0.1	<LOQ
N,O-Didesmethyl tramadol	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Trimethoprim	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
3-Desmethyl trimethoprim	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
5-(3,4,5-Trimethoxybenzoyl)-2,4-pyrimidinediamine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Valsartan	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Valeryl-4-hydroxyvalsartan	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Valsartanic acid	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Venlafaxine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
N-Desmethyl venlafaxine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
O-Desmethyl venlafaxine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
N,O-Desmethyl venlafaxine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Xipamide	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

Table C.17: Mean concentrations determined over 3 replicates for the Rhine and Saar breams with their 95% confidence interval

	Concentration [ng/g]												
	Koblenz 2014 (n=3)		Koblenz 2015 (n=3)			Koblenz 2016 (n=3)		Bimmen 2015 (n=3)			Rehlingen 2015 (n=3)		
Analyte	Fillet [ng/g]	Liver [ng/g]	Fillet [ng/g]	Liver [ng/g]	Plasma [ng/mL]	Fillet [ng/g]	Liver [ng/g]	Fillet [ng/g]	Liver [ng/g]	Plasma [ng/mL]	Fillet [ng/g]	Liver [ng/g]	Plasma [ng/mL]
Amisulpride	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Bezafibrate	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Bicalutamide	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Candesartan	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Carbamazepine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
2-Hydroxycarbamazepine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
3-Hydroxycarbamazepine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
10,11-Dihydro-10-hydroxycarbamazepine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
10,11-Dihydroxy-10,11-dihydrocarbamazepine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Acridone	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Cetirizine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Chlorothiazide	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Citalopram	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Desmethylcitalopram	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Didemethylcitalopram	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Clopidogrel	0.33 ± 0.07	<LOQ	0.5 ± 0.2	<LOQ	<LOQ	0.39 ± 0.04	<LOQ	<LOQ	<LOQ	<LOQ	1.7 ± 0.1	1.0 ± 0.2	0.08 ± 0.01
Clopidogrel carboxylic acid	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Diclofenac	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.41 ± 0.08
Diphenhydramine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.7 ± 0.2	<LOQ
N-Desmethyl diphenhydramine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

	Concentration [ng/g]												
	Koblenz 2014 (n=3)		Koblenz 2015 (n=3)			Koblenz 2016 (n=3)		Bimmen 2015 (n=3)			Rehlingen 2015 (n=3)		
Analyte	Fillet [ng/g]	Liver [ng/g]	Fillet [ng/g]	Liver [ng/g]	Plasma [ng/mL]	Fillet [ng/g]	Liver [ng/g]	Fillet [ng/g]	Liver [ng/g]	Plasma [ng/mL]	Fillet [ng/g]	Liver [ng/g]	Plasma [ng/mL]
Fexofenadine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Flecainide	<LOQ	<LOQ	<LOQ	<LOQ	0.1 ± 0.01	<LOQ	<LOQ	<LOQ	<LOQ	0.123 ± 0.006	0.52 ± 0.06	4.8 ± 0.7	0.63 ± 0.08
Flecainide-meta-O-dealkylated	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Fluconazole	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Fluoxetine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Norfluoxetine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Furosemide	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Gabapentin lactam	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	2.5 ± 0.4	<LOQ
Hydrochlorothiazide	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Lamotrigine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Lidocaine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Norlidocaine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	2.9 ± 0.8	<LOQ	3.0 ± 0.9	<LOQ	<LOQ	2.7 ± 0.7	<LOQ	<LOQ
Metoprolol	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
O-Desmethyl metoprolol	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Oxazepam	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Phenytoin	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Pregabalin lactam	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Primidone	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Quetiapine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Quetiapine sulfoxide	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Sertraline	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Sitagliptin	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Sulfamethoxazole	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
N-Acetyl sulfamethoxazole	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

	Concentration [ng/g]												
	Koblenz 2014 (n=3)		Koblenz 2015 (n=3)			Koblenz 2016 (n=3)		Bimmen 2015 (n=3)			Rehlingen 2015 (n=3)		
Analyte	Fillet [ng/g]	Liver [ng/g]	Fillet [ng/g]	Liver [ng/g]	Plasma [ng/mL]	Fillet [ng/g]	Liver [ng/g]	Fillet [ng/g]	Liver [ng/g]	Plasma [ng/mL]	Fillet [ng/g]	Liver [ng/g]	Plasma [ng/mL]
Sulpiride	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Telmisartan	<LOQ	1.0 ± 0.3	<LOQ	<LOQ	<LOQ	<LOQ	1.1 ± 0.4	<LOQ	1.63 ± 0.09	<LOQ	<LOQ	8.9 ± 0.8	<LOQ
Torasemide	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Hydroxytorasemide	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Tramadol	0.5 ± 0.2	<LOQ	0.7 ± 0.3	<LOQ	<LOQ	0.7 ± 0.4	<LOQ	0.5 ± 0.2	<LOQ	<LOQ	1.26 ± 0.08	2.0 ± 0.8	<LOQ
O-Desmethyl tramadol	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
N-Desmethyl tramadol	0.8 ± 0.5	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.5 ± 0.5	<LOQ	<LOQ	1.0 ± 0.2	3.1 ± 0.4	<LOQ
N,O-Didesmethyl tramadol	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Trimethoprim	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
3-Desmethyl trimethoprim	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
5-(3,4,5-Trimethoxybenzoyl)-2,4-pyrimidinediamine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Valsartan	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Valeryl-4-hydroxyvalsartan	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Valsartanic acid	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Venlafaxine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
N-Desmethyl venlafaxine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
O-Desmethyl venlafaxine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
N,O-Desmethyl venlafaxine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Xipamide	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

Table C.18: p-values (two tails) of the correlation between liver and fillet concentrations for the analytes which were measured in liver and fillet at concentrations >LOQ in more than 4 samples (over all sampling sites)

Analytes	p-value
Acridone	0.78 (n=5)
Carbamazepine	0.011 (n=12)
Clopidogrel	0.32 (n=11)
Diclofenac	0.000005 (n=16)
Diphenhydramine	0.39 (n=15)
Flecainide	0.00052 (n=17)
Lidocaine	0.14 (n=10)
Norlidocaine	0.0071 (n=11)
Tramadol	0.00000024 (n=16)
N-Desmethyl tramadol	0.0023 (n=10)

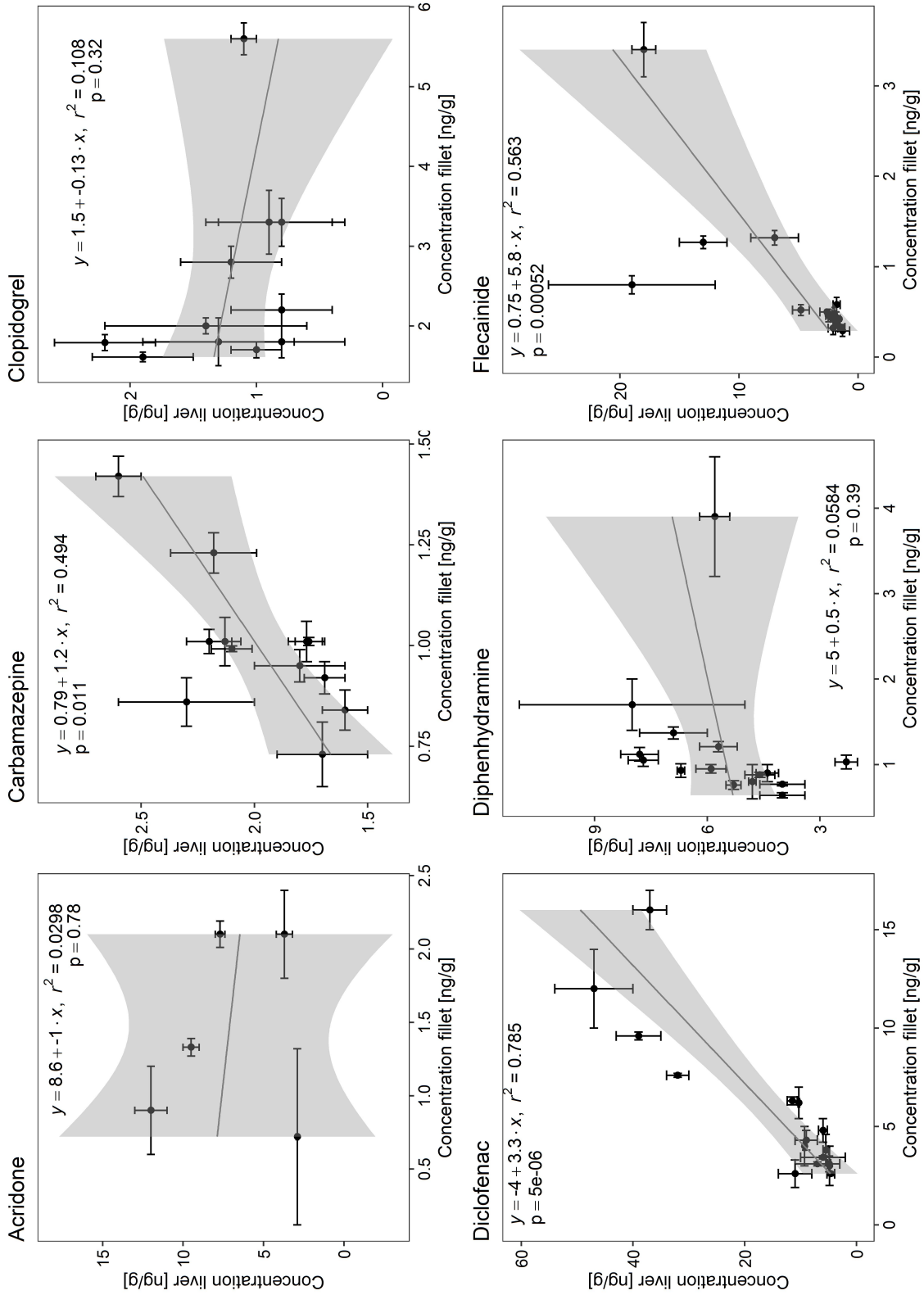

Figure C.7: Relation between fillet and liver concentrations for the analytes detected in both matrices in at least 5 samples. The curves included all samples with detection of the investigated analytes both matrices. Error bars correspond to the 95% confidence interval over triplicate analysis

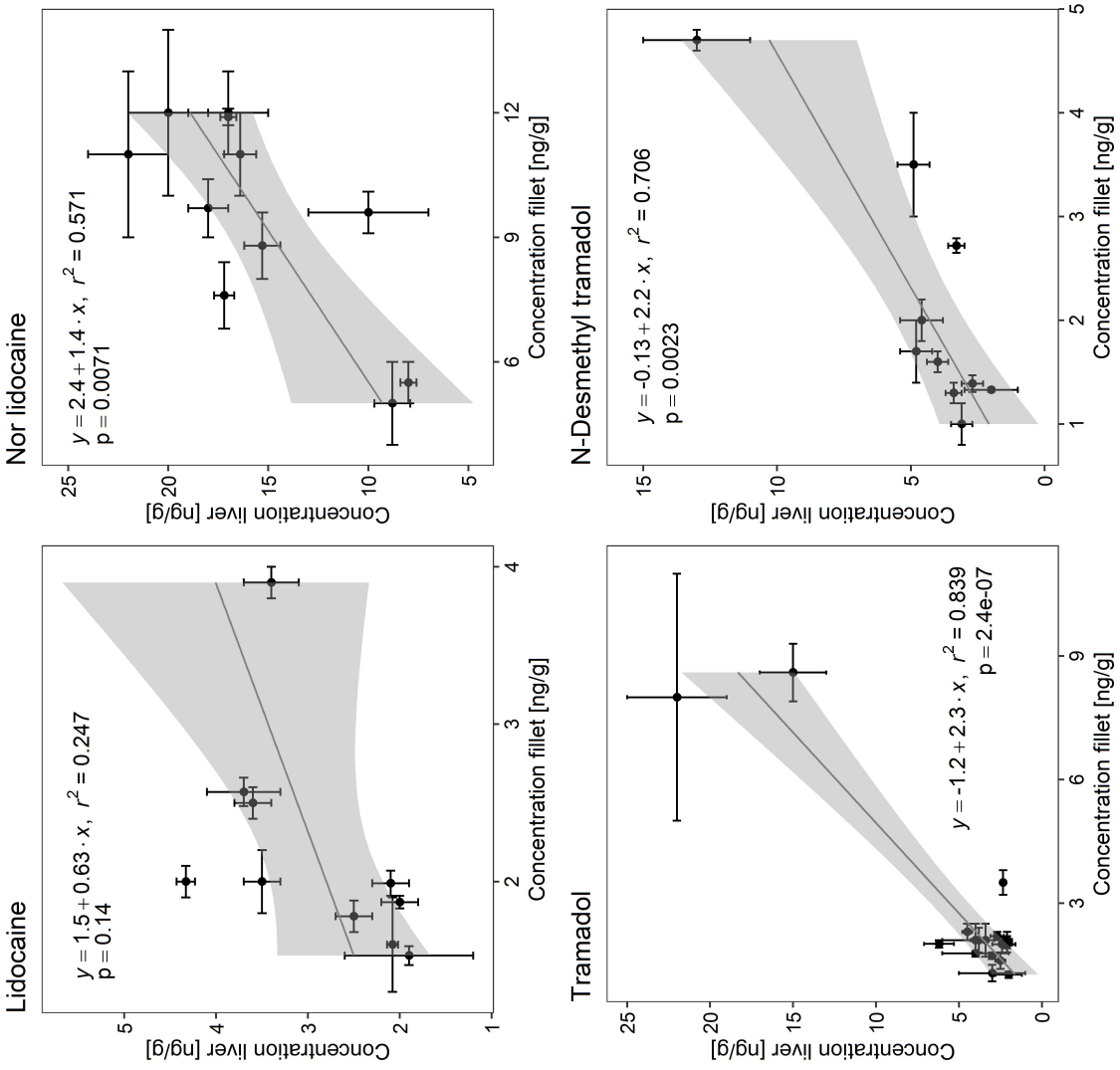

Figure C.7: (continued)

Table C.19: Liver to fillet concentration ratio (determined as slope of the linear regression between both variables) for the analytes showing a correlation between their fillet and liver concentrations

Analytes	Log D at pH 7	Charge	Liver to fillet concentrations ratio
N-Desmethyl tramadol	-0.664	Positive	2.21
Tramadol	0.239	Positive	2.28
Norlidocaine	0.52	Positive	1.37
Flecainide	0.663	Positive	5.84
Diclofenac	1.368	Negative	3.34
Carbamazepine	2.766	Neutral	1.20

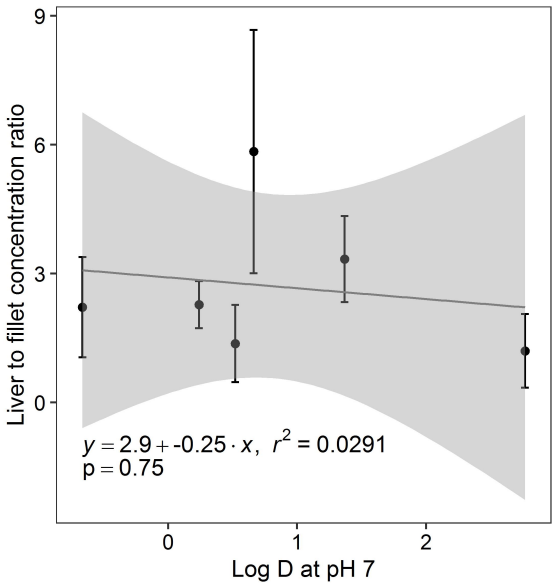

Figure C.8: Relation between liver to fillet concentrations ratio (determined as slope of the linear regression between both variables) and log D at pH 7 for the analytes who show a significant correlation between their fillet and liver concentrations. Error bars correspond to the 95% confidence interval over the slope from the liver to fillet ratio determination.

Table C.20: Correlation between measured concentrations and lipid content of the samples for the Teltow Canal fillet tissues, n correspond to the number of positive detections. p-values (two tails) were determined for all analytes with at least four positive detections

Analytes	p-values
Carbamazepine	0.32 (n=11)
10,11-Dihydroxy-10,11-dihydrocarbamazepine	0.16 (n=10)
Clopidogrel	0.06 (n=11)
Diclofenac	0.79 (n=11)
Diphenhydramine	0.05 (n=11)
Flecainide	0.52 (n=11)
Lidocaine	0.23 (n=11)
Norlidocaine	0.56 (n=11)
Tramadol	0.73 (n=11)
N-Desmethyl tramadol	0.10 (n=11)

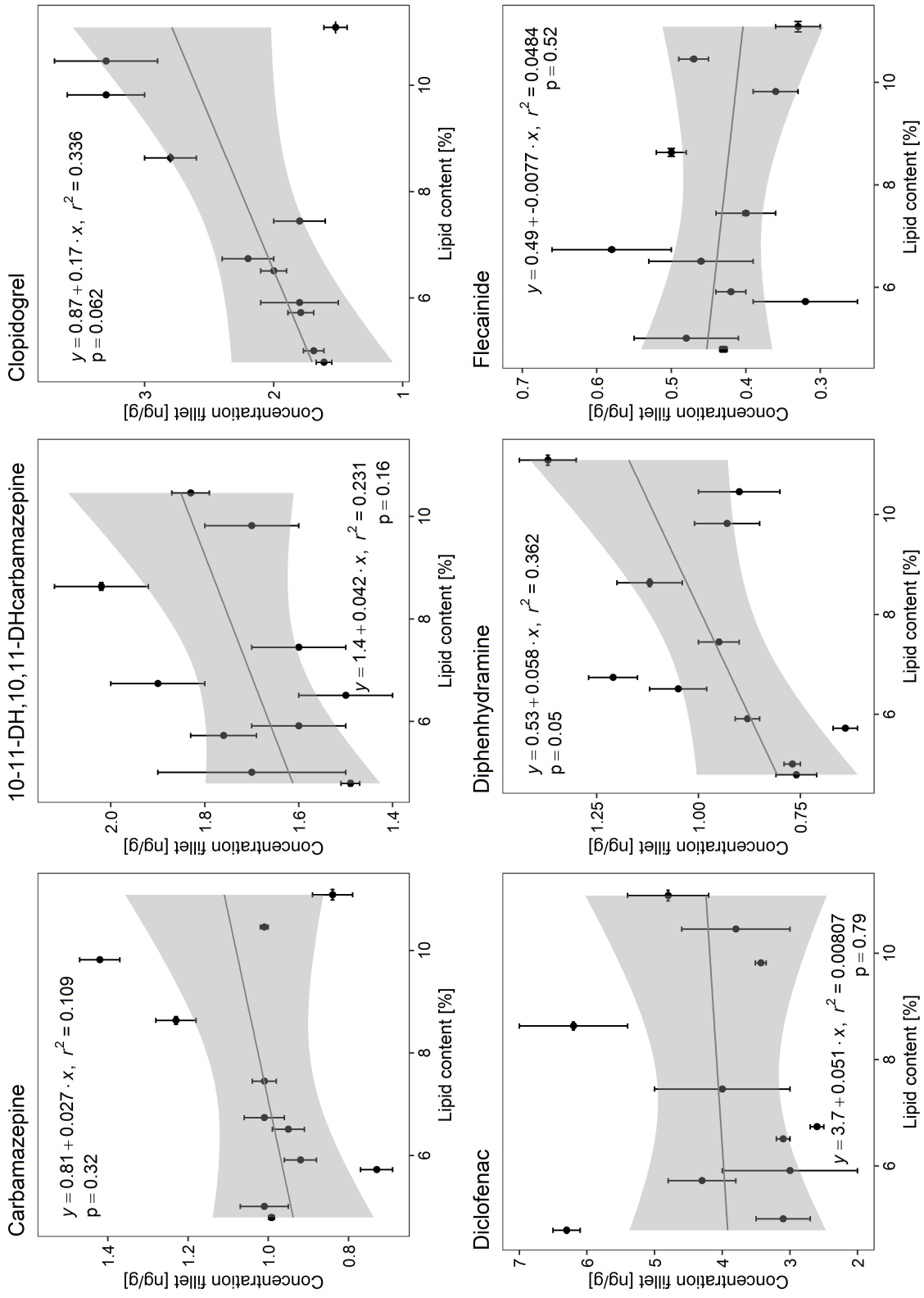

Figure C.9: Relation between fillet concentrations and lipid content for the Teltow Canal samples. All analytes detected in at least 5 samples are represented. Error bars correspond to the 95% confidence interval over triplicate analysis. 10,11-DH-10,11-DHcarbamazepine: 10,11-Dihydroxy-10,11-dihydrocarbamazepine

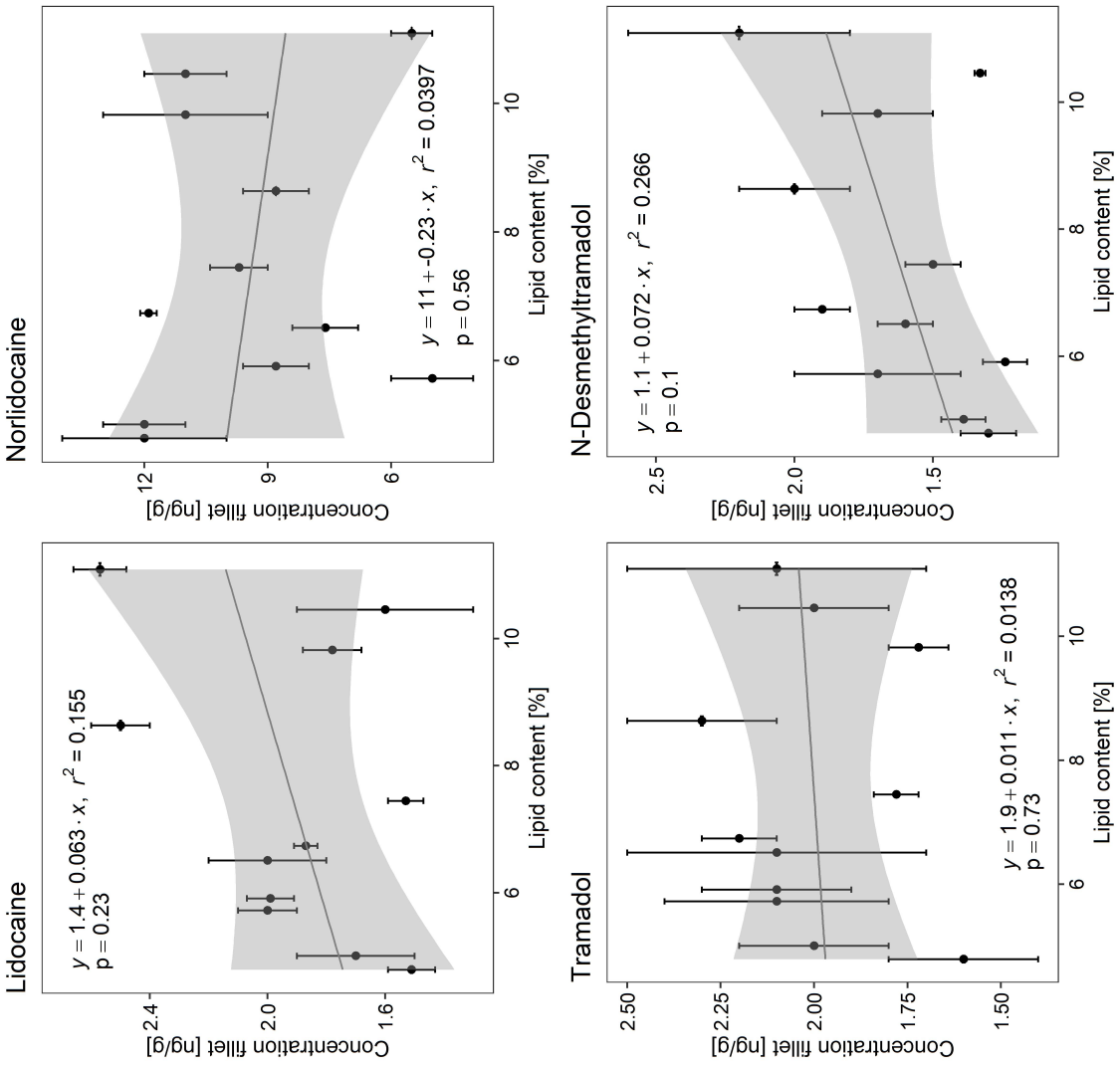

Figure C.9: (continued)

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