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Prioritised Pharmaceuticals in German estuaries and coastal waters: Occurrence and Environmental Risk Assessment

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Highlights:

- A sensitive UHPLC-MS/MS method was developed to determine pharmaceuticals in the ng L^{-1} and sub- ng L^{-1}-range.
- 19 prioritised pharmaceuticals were frequently detected in German coastal regions and their estuaries.
- Riverine inputs of pharmaceuticals, largely reached coastal waters underlining their persistence in brackish and seawaters.
- Clarithromycin and sulfamethoxazole are suspected of posing an ecotoxicological risk in marine and brackish water.

Abstract

In this study a target analysis approach with method detection limits down to 0.01 ng L^{-1} was developed in order to determine ultra-trace pharmaceuticals in seawater of the German coast and their estuaries. The selection of target analytes based on a prioritisation commissioned by the German Environmental Agency considering occurrence in German surface waters, production volumes and ecotoxicological data. Using ultra-high pressure liquid chromatography coupled to a triple quadrupole mass spectrometer equipped with an electrospray ionisation source 21 prioritised pharmaceuticals out of seven therapeutical classes (antibiotics, iodinated X-ray contrast media (ICM), analgesics, lipid reducers, antiepileptics, anticonvulsants, beta-blockers) have been detected in the low to medium ng L^{-1}-range. The most frequently measured substance groups in the German Baltic Sea and German Bight are the ICM, represented by the non-ionic ICM iomeprol (German $Bight_{max}$: 207 ng L^{-1}; Baltic Sea_{max}: 34.5 ng L^{-1}) and the ionic ICM amidotrizoic acid (German Bight: 86.9 ng L^{-1}), respectively. The same pattern of substance distribution could be detected in the German Bight, the German Baltic Sea and their inflows with lower concentrations in the offshore region that are partly a result of dilution with marine water. Pharmaceuticals entering the estuaries and coastal regions are an environmental issue since data on the ecotoxicological effects on aquatic marine organisms is limited. Especially the antibiotics clarithromycin and sulfamethoxazole could be ecotoxicologically / environmentally critical.

Keywords: Pharmaceuticals, UHPLC-MS/MS, seawater, German Bight, Baltic Sea

1. Introduction

The broad range of medicinal products available for human or veterinary use may lead to a global environmental problem (Klatte et al., 2017). Due to the continuous presence of pharmaceuticals entering the aquatic environment through different entry paths they are regarded as a class of pseudo-persistent contaminants (Bu et al., 2016; Daughton, 2003). Pharmaceuticals reach production volumes of up to 100,000 tons per year (aus der Beek et al., 2016), it has been estimated that the spending for the global pharmaceutical market will reach nearly USD 1.5 trillion by 2021, a growing trend in this sector is predicted. Main drivers for this development are market expansion and demographic changes such as growing and aging population (IFPMA, 2017; Roig, 2010; Arnold et al., 2014). Pharmaceuticals go through a strict approval procedure in order to ensure effectiveness and patient safety (Taylor, 2016). But long-term ecotoxicological studies for risk assessment in order to prevent undesirable side effects on the environment have only been rarely considered (Sanderson et al., 2003; Fent et al., 2006; Boxall et al., 2012). Consequently, pharmaceuticals, their metabolites and transformation products reach the environment through different entry paths, such as waste water treatment plants (WWTPs), hospitals and animal husbandry (aus der Beek et al., 2016; Klatte et al., 2017). It is undisputed that the development of pharmaceuticals and their use in medicine is of great value for human society. Nevertheless, their fate is an environmental issue. Besides many diffuse sources such as landfill or manure, a high proportion of the pharmaceuticals is coming from point sources such as WWTPs (including incorrect disposals of unused drugs and hospital sewage). The systems are often not fully capable of processing the sewage and removing the various organic pollutants or lower their concentrations (Heberer, 2002; Verlicchi et al., 2012; Caldwell, 2016). Since only limited data on the occurrence of a variety of pharmaceuticals in the coastal environment is available the need for monitoring pollutants with environmental relevance is indicated (Gaw et al., 2014; Richardson and Ternes, 2014; Arpin-Pont et al., 2016; Pazdro et al., 2016).

The objective of this study is to contribute to the environmental status assessment by providing a dataset with prioritised pharmaceuticals in the German coastal regions and evaluate their ecotoxicological relevance.

Sampling sites in the German Baltic Sea and coastal region of the German Bight in the North Sea and some of its inflows were selected. The Baltic Sea is a 420,000 km² brackish water area with relatively low biodiversity due to its enclosed nature. A transition area is linking the Baltic Sea with the North Sea (HELCOM, 2018). The study area of North Sea is characterised by industrialised and agricultural regions such as the catchments of the rivers Elbe, Weser and Ems which represent some main influxes (Umweltbundesamt, 2014).

Most commonly used for water analysis in the marine and coastal environment is ultra-high pressure liquid chromatography (UHPLC) coupled to massspectrometry equipped with an electrospray ionisation source (ESI), allowing measurements in the low ng L^{-1}-range. Marine and coastal waters require sample pretreatments such as filtration or pH adjustment. Solid phase extraction (SPE) is frequently applied for the enrichment of analytes, sorbents such as Oasis HLB (Waters), Strata-X (Phenomenex) or Chromabond HR-X (Macherey-Nagel) were most commonly used (Wille et al., 2010; Loos et al., 2013; Zhang et al., 2013a; Zhang et al., 2013b; McEneff et al., 2014; Nödler et al., 2014; Borecka et al., 2015; Paiga et al., 2015; Pazdro et al., 2016). For the limited applications in the marine environment mostly Oasis HLB has been used and was also selected as SPE sorbent for our study. Oasis HLB is designed for the enrichment of both polar and non-polar analytes.

In order to evaluate the occurrence and impact of pharmaceuticals, authorities are generating prioritisation lists following certain selection criteria such as consumption/sales, ecotoxicity, elimination rates in WWTPs, occurrence and persistence (Richardson and Ternes, 2011; Sui et al., 2012; Triebskorn et al., 2014; de Voogt et al., 2008; Loos, 2015). In Germany, Bergmann et al. (2011) compiled such a list on behalf of the German Environmental Agency. Based on this prioritised pharmaceutical list a set of substances was

generated, analytically optimised and used for environmental studies in the German coastal region and its estuaries. Table 1 shows the selection of substances and prioritisation criteria such as sales, predicted no effect concentrations (PNECs), maximum measured environmental concentrations (MEC_{max}) and risk quotients (RQs). For more details, refer to Bergmann et al. (2011). Our study focusses on groups of pharmaceuticals such as antibiotics (e.g. macrolide and sulfonamide), analgesics (e.g. diclofenac and paracetamol), ICM (e.g. iomeprol, amidotrizoic acid), lipid-lowering drugs (e.g. bezafibrate), beta blockers (e.g. propranolol), the antiepileptic drug carbamazepine and the anticonvulsant primidone (Supplementary Fig. S1).

Table 1 Analysed compounds and prioritisation criteria based on Bergmann et al. (2011)

Class	Compound	CAS no.	Sales [t year^{-1}]	PNEC* [µg L^{-1}]	MEC_{max}* [µg L^{-1}]	RQs*	Prioritisation*
Antibiotics	Erythromycin	114-07-8	7.8**	2.06E-01	1.700	8.3E+00	P
	Clarithromycin	81103-11-9	13.3**	2.00E-01	0.980	4.9E+00	P
	Roxithromycin	80214-83-1	4.3**	2.00E-01	0.560	2.8E+00	P
	Sulfamethoxazole	723-46-6	26.1**	5.90E-01	1.130	1.9E+00	P
	Sulfamethazine	57-68-1		1.52E-02	4.000	2.6E+02	P
	Sulfadimethoxine	122-11-2		8.80E-03	15.000	1.7E+03	P
	Tiamulin	55297-95-5		3.00E-03	0.200	6.7E+01	P
	Lincomycin	154-21-2		7.00E-02	0.730	1.0E+01	(P)
ICM	Iomeprol	78649-41-9	254.7**				(P)
	Iopamidol	60166-93-0	27.8**				(P)
	Iopromide	73334-07-3	55.8**	6.80E+03	30.000	4.4E-03	(P)
	Amidotrizoic acid	117-96-4	63.99*				(P)
	Ioxitalamic acid	28179-44-4	6.6**				(P)
	Iohexol	66108-95-0	19.9**	1.00E+03	1.500	1.5E-03	(P)
Analgesics	Paracetamol	103-90-2	564.71*	1.00E+00	3.590	3.6E+00	P
	Diclofenac	15307-86-5	84.4**	1.00E-01	3.100	3.1E+01	P
Lipid reducer	Bezafibrate	41859-67-0	12.2**	1.20E+00	5.000	4.2E+00	(P)
Antiepileptic	Carbamazepine	298-46-4	52.3**	2.50E+00	6.100	2.4E+00	P
Anticonvulsant	Primidone	125-33-7	7.21*	3.20E-01	1.100	3.4E+00	P
Beta-Blocker	Nadolol	42200-33-9		2.00E-02	0.180	9.0E+00	(P)
	Propranolol	525-66-6		1.00E-01	0.590	5.9E+00	(P)

*Bergmann et al., 2011; Sales (Germany, 2009); P: high prioritisation; (P): medium prioritisation
RQ = MEC_{max} / PNEC; RQ ≥ 1: ecotoxicological relevance indicated; **Umweltbundesamt, 2013; Sales (Germany, 2012)

2. Materials and Methods

2.1 Background information on the selected prioritised pharmaceuticals

The selection of prioritised pharmaceuticals used for this study aimed at covering a broad range of different therapeutically pharmaceutical classes with high detection frequency in surface waters, high volumes of production and ecotoxicological relevance

($RQ = MEC_{Max}/PNEC \geq 1$) (Bergmann et al., 2011).

WWTPs were identified as major source. Their efficiency and the elimination rates are not only strongly dependent on the equipment used in the treatment process, but also the composition of compounds consumed in the catchment area, that for their part are subject to seasonal fluctuation, are of importance (Daughton, 2014; Zietzschmann et al., 2014; Kümmerer, 2008). Seeber and Hoa (2010) reported elimination rates in conventional sewage treatment plants (STPs) ranging from 0% for the ICM iomeprol/iopamidol/iopromide to 100% for paracetamol. In order to enhance the removal efficiency advanced treatment such as ozonation- or activated carbon-stages might be applied. For a Swiss WWTP equipped with a full-scale ozonation reactor Hollender et al. (2009) reported, that a post-ozonation with doses of 0.79 - 1.16 g O_3 g^{-1} dissolved organic carbon (DOC) achieves elimination rates of 85 - 100% for diclofenac, carbamazepine, clarithromycin, sulfamethoxazole and bezafibrate, and <50% for iopromide. Although these additional steps in the WWTP network seem to reduce the release of pharmaceuticals through the effluent into the receiving water bodies, transformation products may be formed which potentially show a higher bioactivity than their parent compounds (Bedner and Maccrehan, 2006; Celiz et al., 2009; Fatta-Kassinos et al., 2011).

For example, intravascular ICM are polar compounds developed for diagnostic applications. 90-100% of the ICM are excreted unmetabolised by the human body within 24 h (Steger-Hartmann et al., 2002; Weissbrodt et al., 2009). Due to their polarity, stability and lack of biodegradability ionic and non-ionic ICM are poorly removed by WWTPs (Hollender et al.,

2009; Miege et al., 2009) resulting in mean concentrations found in receiving creeks and rivers in Germany of 0.49 µg L^{-1} for the non-ionic iopamidol and 0.23 µg L^{-1} for the ionic ICM amidotrizoic acid, respectively (Ternes and Hirsch, 2000). In their study Kormos et al. (2011) investigated biotransformation products of four ICM in the urban water cycle stating that a transformation product of iomeprol (TP687) showed elevated concentrations of up to 500 ± 60 ng L^{-1} in drinking water. Duirk et al. (2011) reported that ICM may be associated with the formation of iodo-acid disinfection by-products. An environmental impact coming from these toxic transformation products cannot be excluded.

Another prioritised group of pharmaceuticals relevant for this study are the antibiotics, used in human and veterinarian medicine. In Germany consumption rates of 571 t for human medicine and 1,826 t for veterinary used antibiotics were reported for the years 2009 and 2011, respectively. Further reports based on the data of 25 European countries show that 71% of the total sales for veterinarian used antibiotics were represented by tetracyclines, penicillins and sulfonamides (Bergmann et al., 2011; ESVAC, 2013). In Germany sulfonamides like sulfamethazine and sulfadimethoxine are widely used in pig farming, recently sulfamethoxazole was also approved for poultry farming until then the application was limited to human medicine (Jekel and Dott, 2013). The volume of veterinary used antibiotics is decreasing from 1,706 t in 2011 to 742 t in Germany in 2016 (BVL, 2017). Especially due to the spreading of multiresistant pathogens in human and veterinarian medicine antibiotics are a major concern (Gullberg et al., 2011; Richardson and Ternes, 2014).

2.2 Chemicals and reagents

The analytical standards as well as their isotopically labelled compounds were purchased in the highest purity available (>98%) from several suppliers (Supplementary Table S7). For each standard a stock solution (concentration β=1 mg mL^{-1}) was prepared using methanol

as solvent. A 10 ng μL^{-1} mixture of all native standards and a 0.2 ng μL^{-1} internal standard (ISTD) mixture were prepared from the stock solutions, respectively. The methanol used as chromatographic mobile phase and as eluent for SPE was hypergrade for LC-MS LiChrosolv® from Merck (Darmstadt, Germany). Ultrapure water (MilliQ-water) was provided by a Milli-Q Integral 5 system (TOC<3 ppb and R=18.2 MΩ) from Merck. Formic acid as eluent additive for LC-MS/MS was supplied by Sigma-Aldrich and hydrochloric acid (HCl) suprapur® by Merck.

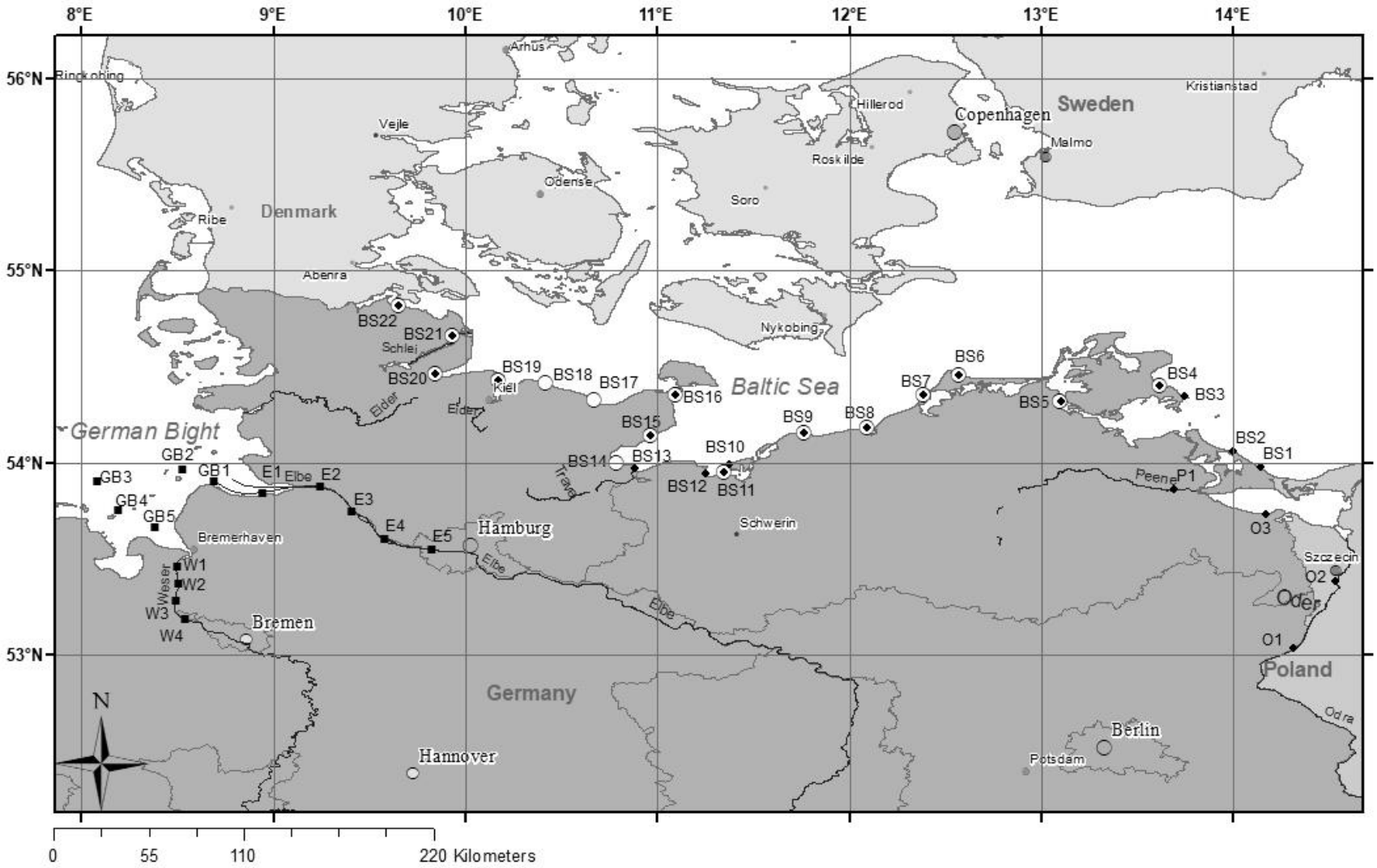
2.3 Sampling sites

Sampling sites are located in the German Bight (North Sea), coastal regions of the Baltic Sea as well as related estuaries and tidal sections of the rivers.

Five water samples in the German Bight, five in the Lower Elbe, and four in the River Weser each 1 L were taken with the research vessel Ludwig Prandtl in May 2015 (except GB3, 5 L). With a maximum distance of 19 nautical miles to the coast GB3 was the most distant sampling site in the German Bight.

In the Baltic Sea campaign three water samples in the River Oder, one in the River Peene and 19 in the Baltic Sea each 2 L were taken in August 2015. This sampling campaign was repeated in March 2016 collecting a total of 16 water samples (each 2 L). In both Baltic Sea campaigns sample sites were in close proximity to the coastline or riverbanks, respectively (Fig. 1).

Fig. 1 Overview of the sampling sites



German Bight (GB), river Elbe (E), river Weser (W), Baltic Sea (BS), river Peene (P1) and river Oder (O):
■ May 2015; ● August 2015; ○ March 2016; (software: Esri ArcGIS Desktop 10.6)

2.4 Sample collection and extraction

Parameters such as salinity, temperature and pH-value were measured at all stations (Supplementary Table S1). Volumes from 1 L to 5 L were stored in precleaned and with the sample twice rinsed amber screw cap bottles. Water samples collected on board during the German Bight campaign were immediately filtered through glass microfiber filters (GF/F, Ø 47 mm, Whatman, GE Healthcare, baked out at 450 °C for 12 h before use). Water samples were enriched via SPE either without pH adjustment ("pH 8", range of 7.5-8.7) or after pH adjustment with HCl ("pH 2"). The extraction was carried out using Oasis HLB cartridges (Hydrophilic-lipophilic-balanced reversed-phase sorbent, 60 µm, 500 mg, 6cc, Waters, Milford, MA, USA). The water samples were spiked with mass-labeled internal standards (absolute: 7.5-56 ng) prior to extraction. Oasis HLB cartridges were conditioned with 3 x 2 mL methanol and equilibrated with 3 x 2 mL MilliQ-water pH 8 or pH 2 (adjusted with HCl), respectively. Water samples were extracted at a flow of 1.5 - 5 mL min^{-1}. After the extraction a washing step followed using the same flow conditions and 3 x 2 mL MilliQ-water pH 8 or pH 2, respectively. Subsequently, the HLB cartridges were dried for at least 1 h with pre-cleaned air. The washed and dried cartridges were kept at 4 °C until elution in the laboratory. The cooled water samples collected in the Baltic Sea were filtered and extracted at the Helmholtz Centre Geesthacht, as described above. All extracts were gained by eluting with 3 x 3 mL methanol. Under a gentle heated nitrogen flow (60 °C) (Flowtherm optocontrol s, Barkey) the extracts were concentrated to 150 µL. MilliQ-water and the injection standard benzotriazole-D4 were added to reach a final volume of 750 µL and an aqueous amount of 80%. The sample extracts were filtrated through a nylon syringe filter (4 mm, 0.2 µm, Nalgene™, Thermo Fisher Scientific, Waltham, MA, USA) prior to UHPLC-MS/MS analysis.

2.5 UHPLC-MS/MS analysis

The analysis was performed using an Agilent Technologies 1290 UHPLC system coupled to an Agilent Technologies 6490 triple quadrupole mass spectrometer operating with the Agilent Jet Stream electron spray ionisation (ESI) source. A ZORBAX Eclipse Plus C18 column (Rapid Resolution High Definition, 1.8 µm, 2.1x150 mm, Agilent Technologies) was used combined with a ZORBAX Eclipse Plus C18 precolumn (1.8 µm, 2.1x5 mm, Agilent Technologies) for the chromatographic separation. An optimised gradient using A: MilliQ-water + 0.1% formic acid (FA) and B: methanol + 0.1% FA as mobile phases at a flow of 0.2 mL min^{-1} was applied. Table S2 (Supplementary) combines further general operating parameters of the chromatographic and the mass spectrometric analysis. All precursor, quantifier and qualifier ions as well as some substance specific ionisation parameters such as the Cell Accelerator Voltage (CAV) are shown in Table S3 (Supplementary).

3. Results and discussion

3.1 Method optimisation

Method optimisation was carried out for sample preparation/extraction and LC-MS/MS. Matrix samples (1 L) such as marine, brackish and fresh water were used for method optimisation (Supplementary Table S4). The environmental samples for pH 8 were not adjusted, they remained unchanged. For pH 2 HCl was added. The samples were spiked with native substances and ISTD showing that pH 8 was suitable for a wide range of pharmaceuticals used in this study. Acidification with HCl was necessary to adjust a pH-value of 2 especially for the extraction of ionic compounds such as the ionic ICM ioxitalamic acid and amidotrizoic acid. Besides the recoveries, the chromatographic appearance of the peaks (quantifier and qualifier) was taken into consideration (e.g. peak shape, signal intensity) before defining pH-values for solid phase extraction. In order to verify the suitability of the applied extraction flow, the extraction efficiency was examined using a minimum flow

of 2.5 mL min^{-1} and a maximum flow of 7 mL min^{-1}. Results showed no significant difference in the tested extraction flows (Supplementary Table S5). Sample extracts were evaporated after elution with methanol to a final volume of 150 µL. Evaporating the extracts to a final volume of 50 µL or to dryness result in analyte and matrix residues that could not be completely redissolved in the vial.

Methanol, acetonitrile and water as mobile phase constituents were used for the optimisation of the LC-separation. Formic acid, ammonium acetate and ammonium formate were tested at different concentrations as mobile phase additives. Optimum results were obtained with A: water+0.1% FA and B: methanol+0.1% FA (Supplementary Table S2).

Operating parameters for the MS-detection such as sheath gas temperature, sheath gas flow, drying gas temperature, drying gas flow, nebulizer, ion funnel parameters and capillary voltage were optimised and shown in Table S2 (Supplementary).

Prior to injection the environmental samples were filtered through a nylon syringe filter in order to remove particles larger than 0.2 µm. Different syringe filter materials such as nylon, regenerated cellulose and polypropylene were tested. A defined volume of a standard solution was filtered through the syringe filters and the recovery was calculated, showing stronger retention of pharmaceuticals for regenerated cellulose and polypropylene.

3.2 Method performance and validation

Calculation of the limit of detection (LOD) and limit of quantification (LOQ) was performed with equation (1) for the LOD and (2) for the LOQ. The limits of detection and quantification were determined for the instrument as well as for the method. For the instrument detection limit (IDL) and the instrument quantification limit (IQL) ten blank injections (20% methanol + 80% MilliQ-water) were measured; the mean value ($mean_{Blank}$) was calculated as well as the standard deviation (s).

(1) $LOD = 3s + mean_{Blank}$

(2) $LOQ = 10s + mean_{Blank}$

The same approach was used for the method detection limits (MDL) and quantification limits (MQL) using blank samples represented by MilliQ-water processed in accordance with the environmental samples. If this approach was not applicable the signal-to-noise-ratio (S/N) of 3 and 9 was used for evaluation of the detection and quantification limits, respectively. In case of LODs and LOQs this was achieved by extrapolation of the lowest applicable analyte concentration. The method quantification limits varied from 0.01 - 0.4 ng L^{-1} for most of the analytes except to some ICM (iohexol 0.53 ng L^{-1} - iomeprol 3.29 ng L^{-1}), sulfamethazine (1.09 ng L^{-1}), carbamazepine (1.12 ng L^{-1}), diclofenac (0.61 ng L^{-1}) and paracetamol (0.46 ng L^{-1}) (Supplementary Table S6). Further method development was conducted measuring the signal suppression, repeatability and recovery. The method precision under repeatability conditions has been in the range of 1-19%, except ioxitalamic acid (33%). Recoveries in different water matrices calculated with the internal standard method are given in Table S4 (Supplementary). The comparison of the validation parameters with other studies show similar MQLs and recovery rates (Loos et al., 2013; Zhang et al., 2013b; Nödler et al., 2014; Pazdro et al., 2016).

For the quantification, calibration solutions were prepared in accordance with the environmental samples by adding the same amount of injection standard and ISTD,

adjusting to a final volume of 750 µL with an aqueous amount of 80%. Calibration curves were set up in concentration ranges of 0.01 - 100 pg μL^{-1} (11-point calibration) and 0.01 - 500 pg μL^{-1} (13-point calibration). For each substance a linear calibration curve with a weighing factor of 1/x and a regression coefficient $R^2>0.99$ was obtained. The software MassHunter B06.00 QQQ Quantitative Analysis was used for the batch evaluation. Table S7 (Supplementary) contains all native substances with the assigned ISTD. For some native compounds mass-labelled standards were not available, in this case other ISTD, often out of the same pharmaceutical class, were selected. Bracketing calibration was applied for the sample batches. The sequence calibration included all calibration levels at the beginning and in end of the sequence. In addition each sample batch included blanks and quality standard controls to control carryover and check the stability of the detection system. The optimised method is robust with acceptable reproducibility and suitable for quantification.

3.3 Occurrence of pharmaceuticals in coastal and river water in comparison to other studies

In our study 19 out of 21 prioritised pharmaceuticals were frequently detected in the coastal regions and their estuaries, except paracetamol and nadolol. Substance pattern of classes of pharmaceuticals in coastal regions are clearly related to the respective patterns in the estuaries and rivers (Fig. 2 and 3) with decreasing concentrations towards the coastal regions mainly due to mixing with lower contaminated seawater. In addition, a high pattern similarity was observed for the investigated coastal areas of the German Baltic Sea and the German Bight. Generally, this applies also for individual substances (Supplementary Table S8).

Typical sum concentrations for the measured pharmaceuticals were 150 - 1000 ng L^{-1} in rivers, 10 - 100 ng L^{-1} and 20 - 500 ng L^{-1} in estuaries and coastal regions of the Baltic Sea and German Bight, respectively. The compounds could be detected offshore, e.g. at station GB3 (19 nautical miles offshore, 30 psu), indicating that degradation in brackish and seawater or sinks like binding to suspended matter or sediments is limited (Supplementary Table S8).

Fig. 2 and 3 show that the ICM are the dominant pharmaceutical class in the German Bight, the Baltic Sea and its surrounding inflows, iomeprol and iopromide being the main components. Concentrations for iomeprol reached up to 488 ng L^{-1} in the Weser river at station W3, up to 34.5 ng L^{-1} in the Baltic Sea (BS5) and 207 ng L^{-1} in the German Bight (GB5).

Fig. 2 Concentrations of pharmaceuticals [ng L^{-1}]: German Bight and the rivers Elbe and Weser (May 2015)

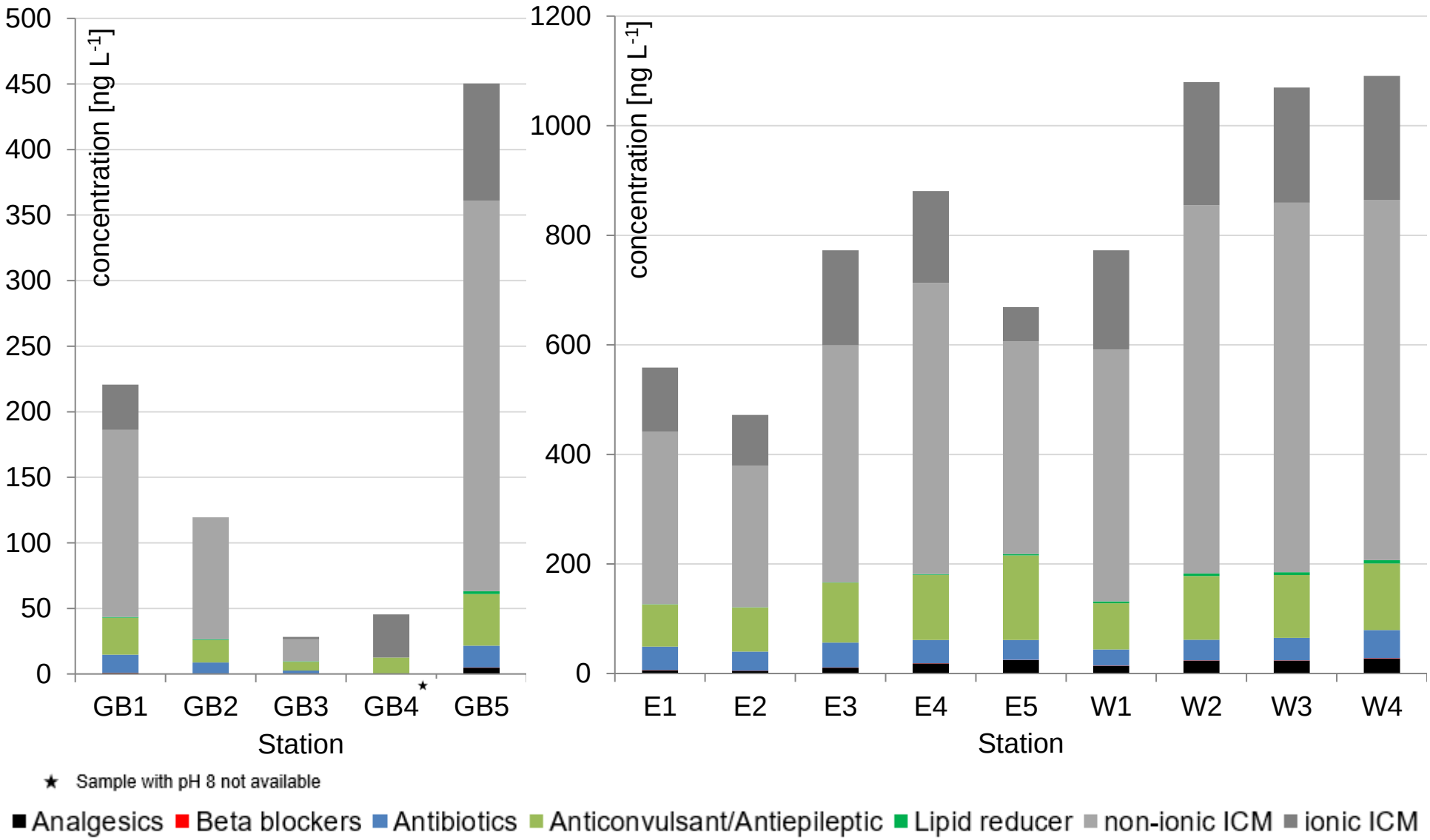
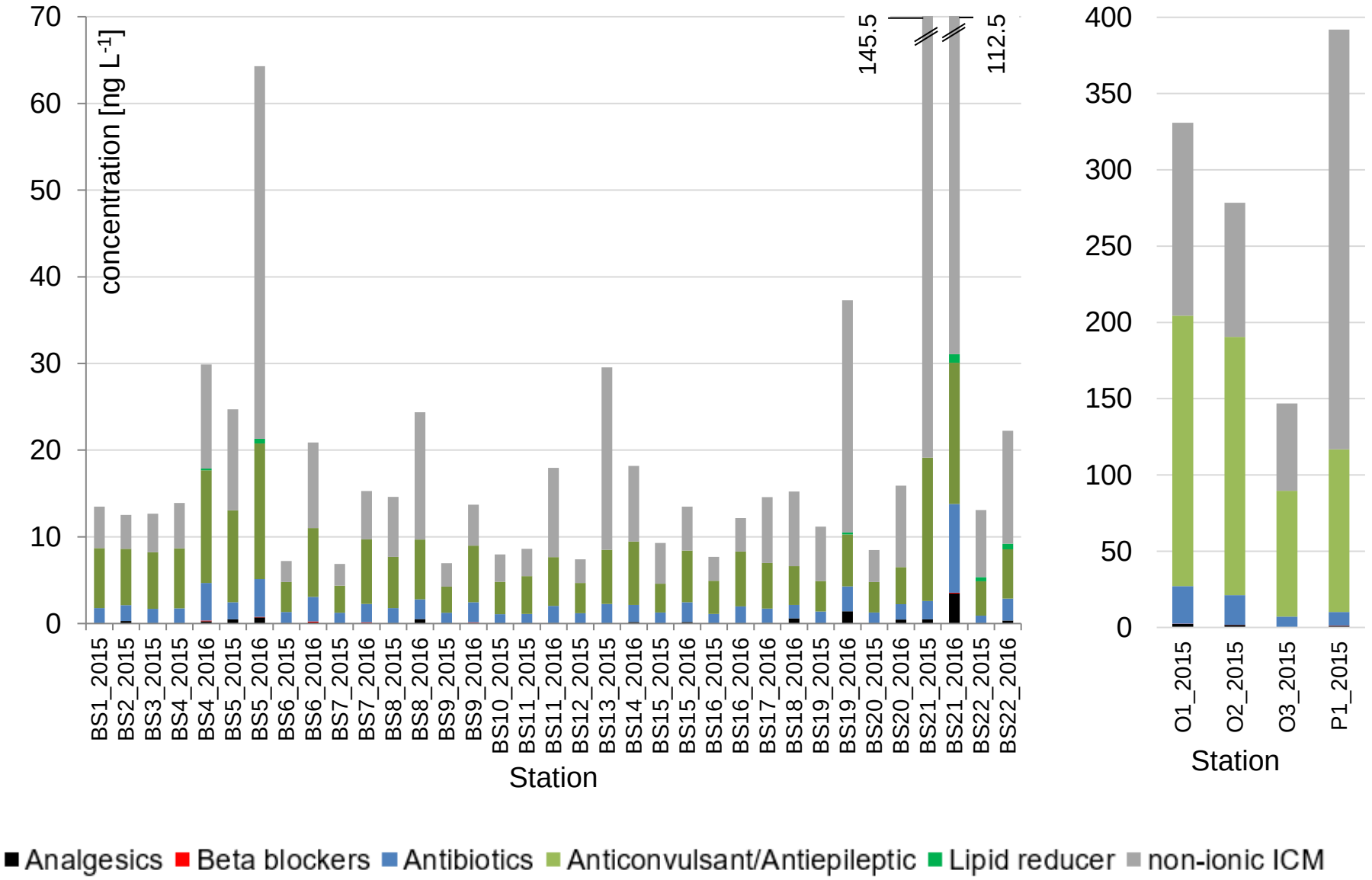


Fig. 3 Concentrations of pharmaceuticals [ng L^{-1}]: Baltic Sea and rivers Oder and Peene (August 2015; March 2016)



The antiepilectic drug carbamazepine also being one of the main compounds in the observed pattern showed concentrations up to 162 ng L^{-1} in the Oder river (O1). Concentrations in the German Bight ranging between 4.8 ng L^{-1} (GB3) and 29.7 ng L^{-1} (GB5) correspond to the findings of the Federal Maritime and Hydrographic Agency of Germany, reporting similar levels between 17 ng L^{-1} in the estuary and 2 ng L^{-1} in offshore regions (Bundesamt für Schifffahrt und Hydrographie (BSH), 2014).

Primidone out of the pharmaceutical class of the anticonvulsants could be found in the Baltic Sea with concentrations ranging between 2.3 ng L^{-1} (BS20) - 6.7 ng L^{-1} (BS5), corresponding to the findings in seawater documented in the status report of the UNESCO and HELCOM (2017) with a maximum value of 5.8 ng L^{-1} in the Baltic Sea and to the concentrations detected in this study in the German Bight (1.9 ng L^{-1} (GB3) - 9.7 ng L^{-1} (GB5)). The surface water of the rivers Oder, Peene, Schlei, Weser and Elbe show higher concentrations up to 49.1 ng L^{-1} (E5).

The group of antibiotics was dominated by the sulfonamide sulfamethoxazole, accounting for nearly 80% of antibiotic sum concentrations in the Baltic Sea and the German Bight. Highest concentrations were observed in the Elbe river (42.6 ng L^{-1}, E3) and 3.29 ng L^{-1} and 13.0 ng L^{-1} in the Baltic Sea (BS4) and German Bight (GB5), respectively.

The macrolides represented by erythromycin, clarithromycin and roxithromycin were detectable in nearly every water sample of the German Bight even 19 nautical miles offshore at station GB3 with concentrations of 0.13 ng L^{-1} (erythromycin), 0.41 ng L^{-1} (clarithromycin) and 0.09 ng L^{-1} (roxithromycin). Out of this antibiotic group erythromycin and clarithromycin are included on the first ‘watch list’ in the Commission Implementing Decision (EU) 2015/495 (European Commission, 2015).

Generally, surveys on the occurrence of pharmaceuticals in marine waters are scarce compared to studies in other surface waters, especially rivers as well as WWTPs. In addition, from Table S9 (Supplementary), it is obvious that there is a minimal overlap of active

substances investigated between our study and those of other authors. Most studies for marine waters focus only on a few selected compounds such as carbamazepine, diclofenac, or sulfamethoxazole.

In the studies comprising ICM (Baltic Sea, Aegean Sea & Dardanelles, Nödler et al., 2014; Rhine river, Singer et al., 2009) the ICM were the dominating compound class, matching our findings. As in our investigations, carbamazepine generally was found at comparably high concentrations. A further detailed comparison is complicated not only by the minor overlap in the analysed substances but also due to different areas of investigation.

3.4 Evaluation of ecotoxicological relevance

Risk quotients (RQ) were calculated as quotients of maximum measured environmental concentrations (MEC) and predicted no effect concentrations (PNEC), the latter based on ecotoxicological data and safety (assessment) factors (European Medicines Agency (EMEA), 2018, European Commission, 2003). RQs were classified as medium risk for $0.1 \leq RQ < 1$ and as high risk for $RQ \geq 1$ (Hernando et al., 2006; Verlicchi et al., 2012). Publically available ecotoxicity data for pharmaceuticals on the European market are scarce, and often derived from few acute toxicity data mostly for a very limited number of freshwater species (BIO Intelligence Service, 2013). For our assessment a distinction was made between freshwater and brackish/marine waters.

Table 2 summarises the results for freshwater. RQs were calculated with PNECs taken from Bergmann et al. (2011). None of the investigated pharmaceuticals were classified as high risk; sulfamethazine, diclofenac and primidone as medium risk for freshwater organisms. The safety factors applied by Bergmann et al. (2011) ranged from 10 to 25,000 due to the availability of ecotoxicological data. Generally, the uncertainty of the assessments largely depend on the data available for the derivation of PNECs. E.g. for carbamazepine, Triebskorn et al. (2007) estimated a LOEC (lowest observed effect concentration) of

1 µg L^{-1} resulting from cellular effects in kidneys of exposed fish. This raises the question if carbamazepine in our study should be classified as high risk pharmaceutical (RQ 1.6, based on a safety factor of 10).

Table 2 Environmental risk assessment - freshwater (<1 psu)

Compound	Effect conc.* [µg L^{-1}]	Safety factor*	PNEC* [µg L^{-1}]	MECmax [µg L^{-1}]	RQmax
Erythromycin	1.03E+01	50	2.06E-01	3.90E-03	1.89E-02
Clarithromycin	2.00E+00	10	2.00E-01	8.07E-03	4.04E-02
Roxithromycin	1.00E+03	5000	2.00E-01	3.64E-03	1.82E-02
Sulfamethoxazole	5.90E+00	10	5.90E-01	4.25E-02	7.20E-02
Sulfamethazine	3.81E+02	25000	1.52E-02	1.74E-03	1.14E-01
Sulfadimethoxine	4.40E+01	5000	8.80E-03	4.90E-04	5.57E-02
Tiamulin	3.00E+00	1000	3.00E-03	1.10E-04	3.67E-02
Lincomycin	7.00E+01	1000	7.00E-02	5.40E-04	7.71E-03
Iomeprol				4.88E-01	
Iopamidol				6.05E-02	
Iopromide	6.80E+04	10	6.80E+03	1.24E-01	1.82E-05
Amidotrizoic acid				2.22E-01	
Ioxitalamic acid				4.11E-03	
Iohexol	1.00E+05	100	1.00E+03	3.92E-02	3.92E-05
Paracetamol	1.00E+03	1000	1.00E+00	n.d.	
Diclofenac	1.00E+00	10	1.00E-01	2.71E-02	2.71E-01
Bezafibrate	6.00E+03	5000	1.20E+00	6.02E-03	5.02E-03
Carbamazepine	2.50E+01	10	2.50E+00	1.62E-01	6.48E-02
Primidone	1.60E+01	50	3.20E-01	4.91E-02	1.53E-01
Nadolol	1.00E+02	5000	2.00E-02	1.00E-05	5.00E-04
Propranolol	1.00E+00	10	1.00E-01	7.00E-04	7.00E-03

0.1≤RQ<1: medium risk; *Bergmann et al. (2011)

For brackish and marine water RQs were calculated separately. The results of the assessment are summarised in Table 3. The compilation of gathered ecotoxicological data for brackish and marine species is given in Table S10 (Supplementary). Safety factors were applied according to European Commission (2003). For the investigated brackish as well as marine waters erythromycin was classified as medium risk, clarithromycin and sulfamethoxazole as high risk pharmaceuticals. Due to the lack of adequate data for different trophic levels of brackish/marine species, high safety factors of 10,000 had to be applied resulting in a high uncertainty of the risk characterisation.

Table 3 Environmental risk assessment - brackish (1-10 psu) and marine (>10 psu) water samples

Compound	Organism	Endpoint	Duration	Effect conc. [µg L^{-1}]		Reference	Marine MECmax [µg L^{-1}]	Brackish MECmax [µg L^{-1}]	Safety factor	PNEC [µg L^{-1}]	Marine RQ	Brackish RQ
Erythromycin	*Anabaena sp CPB4337*	luminescence	72 h	**2.20E+01**	(EC50)	González-Pleiter et al. (2013)	9.40E-04	2.00E-03	10000	2.2E-03	4.3E-01	9.1E-01
Clarithromycin	*Skeletonema marinoi*	growth	72 h	**1.52E-01**	(EC50)	Minguez et al. (2016)	1.66E-03	1.71E-03	10000	1.5E-05	1.1E+02	1.1E+02
Roxithromycin	/	/	/	/		/	4.70E-04	4.50E-04				
Sulfamethoxazole	*Synechococcus leopoliensis*	growth	96 h	**5.90E+00**	(NOEC)	Ferrari et al. (2004)	1.30E-02	3.92E-02	10000	5.9E-04	2.2E+01	6.6E+01
Sulfamethazine	*Vibrio fischeri*	luminescence	5-30 min	**>1.00E+05**	(EC50)	Białk-Bielinska et al. (2011)	8.70E-04	2.30E-03	10000	1.0E+01	8.7E-05	2.3E-04
Sulfadimethoxine	*Artemia nauplii*	lethal	96 h	**1.95E+04**	(LC50)	Migliore et al. (1993)	1.30E-04	4.70E-04	10000	2.0E+00	6.7E-05	2.4E-04
Tiamulin	/	/	/	/		/	1.00E-04	7.00E-05				
Lincomycin	*Synechococcus leopoliensis*	growth	96 h	**7.80E+01**	(NOEC)	Andreozzi et al. (2006)	2.80E-04	4.40E-04	10000	7.8E-03	3.6E-02	5.6E-02
Iomeprol	/	/	/	/		/	2.07E-01	4.84E-01				
Iopamidol	/	/	/	/		/	3.16E-02	5.95E-02				
Iopromide	/	/	/	/		/	3.41E-02	9.02E-02				
Amidotrizoic acid	/	/	/	/		/	8.69E-02	2.21E-01				
Ioxitalamic acid	/	/	/	/		/	2.35E-03	3.79E-03				
Iohexol	/	/	/	/		/	2.55E-02	3.90E-02				
Paracetamol	Artemia salina	immobilisation	48 h	**>1.00E+05**	(EC50)	Minguez et al. (2016)	5.70E-04	n.d.	10000	1.0E+01	5.7E-05	
Diclofenac	Synechococcus leopoliensis	growth	96 h	**1.00E+04**	(NOEC)	Ferrari et al. (2004)	4.82E-03	2.34E-02	10000	1.0E+00	4.8E-03	2.3E-02
Bezafibrate	Anabaena sp CPB4337	luminescence	24 h	**7.62E+03**	(EC50)	Rosal et al. (2010)	2.06E-03	4.71E-03	10000	7.6E-01	2.7E-03	6.2E-03
Carbamazepine	Cyclotella meneghiniana	growth	96 h	**>1.00E+04**	(NOEC)	Ferrari et al. (2004)	2.97E-02	8.76E-02	10000	1.0E+00	3.0E-02	8.8E-02
Primidone	/	/	/	/		/	9.73E-03	2.88E-02				
Propranolol	Cyclotella meneghiniana	growth	96 h	**9.40E+01**	(NOEC)	Ferrari et al. (2004)	2.20E-04	5.00E-04	10000	9.4E-03	2.3E-02	5.3E-02

0.1≤RQ<1: medium risk RQ≥1: high risk

4. Conclusions

A validated target analysis for the trace level determination of 21 prioritised pharmaceuticals out of seven different therapeutical classes was successfully applied to investigate surface waters of the German Bight, the German Baltic Sea and major contributing estuaries and rivers. The observed similarity of the substance pattern revealed the inputs through riverine and estuarine waters to the corresponding coastal areas as well as the relative persistence of the investigated pharmaceuticals under brackish and seawater conditions.

The dominating class of active compounds were the ICM with the main components iomeprol and iopromide; other principal components were the antiepileptic drug carbamazepine, the anticonvulsant primidone, and the antibiotic sulfamethoxazole.

The occurrence and persistence of ICM in WWTPs and rivers is well studied and documented in several publications (Singer et al., 2009; Echeverria et al., 2013; Kormos et al., 2011; Perez and Barcelo, 2007). In addition, our findings support the low degradability of iomeprol, iopamidol, iopromide and amidotrizoic acid as well in brackish and seawater.

For the investigated German rivers, none of the analysed pharmaceuticals were classified as high risk; sulfamethazine, diclofenac and primidone as medium risk for freshwater organisms. For the investigated brackish as well as marine waters erythromycin was classified as medium risk, clarithromycin and sulfamethoxazole as high risk pharmaceuticals. Due to the lack of adequate data especially for different trophic levels of brackish/marine species, high safety factors of 10,000 had to be applied resulting in a high uncertainty of the risk characterisation. To further evaluate the ecotoxicity of the investigated pharmaceuticals and to estimate whether the observed concentrations may have an effect on marine ecosystems, an improved data situation on marine test organisms is needed. These environmental concerns are also addressed in the directive 2013/39/EU of the European parliament and of the council, demanding a documentation and monitoring of pharmaceuticals and their residues in the environment.

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Appendix A. Supplementary

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Notes

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