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# Contamination of the southern Baltic Sea waters by the residues of selected pharmaceuticals: Method development and field studies

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ABSTRACT

In this study the occurrence of thirteen pharmaceuticals in seawaters collected from southern Baltic Sea was evaluated for the first time. It was performed by applying newly developed analytical procedure. The method was characterized in terms of its basic validation parameters as well as matrix effects, extraction efficiency and absolute recovery. The results were expressed as *result ± expanded uncertainty*, which was estimated according to the Guide to the Expression of Uncertainty in Measurement. Additionally, in order to verify the influence of variable parameters of the analyzed samples on method performance parameters, chemometric analysis was carried out. The obtained results revealed that residues of pharmaceuticals were present in seawaters at a concentration level of ng $L^{-1}$. Trimethoprim, sulfamethoxazole and enrofloxacin were most frequently detected compounds. The highest concentration was determined for ketoprofen (135.0 ± 10.9 ng $L^{-1}$). Marine pollution potential hotspots were found in enclosed or semi-enclosed bodies of water.



## 1. Introduction

Pharmaceuticals are a class of emerging environmental contaminants. The occurrence of these compounds in aquatic ecosystems has received increasing attention in recent years due to their pseudo-persistancy nature (resulting from their continuous discharge into the environment) and potential biological activity. A lot of data has revealed that they can have a negative impact on living organisms and ecosystems (Boxall et al., 2003; Fent et al., 2006; Kümmerer, 2009). Therefore, it is very important to monitor their concentration in the environment. Many studies have demonstrated, that drug residues in treated wastewater and surface water are very widespread (Bendz et al., 2005; Kasprzyk-Hordern et al., 2009; Vulliet and Cren-Olivé, 2011). Still very little is known about the risks posed by these substances to the marine environment. Meanwhile, this is of upmost importance since this milieu is considered to be the final sink of the most persistent compounds. Moreover, inland seas are ecosystems potentially exposed to high pharmaceutical loads. A good example is the Baltic Sea.

The Baltic catchment area is home to about 85 million people and various drugs industry, intensive farming and animal husbandry are located in the surrounding countries. The Baltic Sea is almost entirely land-locked and the water exchange with North Sea is very limited. Its special geographical and oceanographic characteristics render this ecosystem highly susceptible to the environmental impacts of human activities. This may have led to exposure of this region to a constant supply and accumulation of hazardous compounds including pharmaceuticals (HELCOM, 2010).

Apart from our preliminary study on the occurrence of sulfonamides, trimethoprim and enrofloxacin in marine waters (Borecka et al., 2013) and tetracyclines in sediments (Siedlewicz et al., 2014), existing data on the presence of pharmaceuticals in the Baltic Sea is limited to the Bay of Germany. Beck et al. (2005, 2009) determined the occurrence of anti-inflammatory, antiepileptic, lipid regulator and analgesic drugs as well as estrogenic compounds in a concentration range of 0.03–17.2 ng $L^{-1}$. Recently Nödler et al. (2014) analyzed various classes of micropollutants including pharmaceuticals (non-steroidal anti-inflammatories, stimulants, antihypertensives, iodinated X-ray contrast media, antibiotics, lipid regulators, antiallergics, anticonvulsants, sedatives and antidepressants, one gastric acid regulator, one

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antipsychotic and one breast cancer drug) in different marine waters samples including those collected from the Bay to Germany. The maximum concentrations of pharmaceutical residues were in the range from 4.1 to 1159 ng $L^{-1}$.

To enrich our knowledge on the contamination of the Baltic Sea by pharmaceuticals, the aim of this study was to expand our previous research and evaluate the presence of the residues of drugs in the waters collected from the coastal area of the southern Baltic more deeply.

Based on the previously obtained results (Borecka et al., 2013) the list of analytes was modified. As three sulfonamides (sulfamethoxazole, sulfachloropyridazine, sulfisoxazole) chosen in the former study were not present in any of the analyzed marine water samples, they were not included in the present study. However, instead of them five additional compounds – oxolinic acid and four non-steroidal anti-inflammatory drugs (diclofenac, ibuprofen, ketoprofen, naproxen) were added. General information about the pharmaceuticals chosen for this investigation is listed in Table 1. The selected drugs are consumed in large quantities and have been used for many years in human and veterinary medicine. Therefore, the possibility of their occurrence in the environmental samples is very high (Sarmah et al., 2006). This table also includes literature data about their impact on the organisms living in the Baltic Sea. Presented data will allow the evaluation of the ecotoxicological risks of the analyzed compounds basing on the results (the concentration of pharmaceuticals in the marine waters) obtained in this study.

The method used for the determination of pharmaceutical residues in marine waters was based on our previously developed method, where solid phase extraction carried out on SPE disks and liquid chromatography coupled with tandem mass spectrometry in a multiple reaction monitoring mode were used (Borecka et al., 2013). However, as we changed the group of analytes, the previously published method was modified and revalidated. To prove the quality of the obtained results, beside validation parameters of the applied method, additional parameters such as expanded uncertainty, matrix effects, absolute recoveries and extraction efficiency were determined for each analyzed sample. Moreover, for the first time the influence of seawater salinity and the amount of suspended matter of the analyzed seawater samples on the method performance parameters was verified using chemometric analysis (using hierarchical cluster analysis).

As a result in this paper we describe in detail the results obtained from much bigger sampling campaign in comparison to the previous study. Samples were collected from the areas, which receive severe pollution loads and where is a great probability of high concentrations of selected compounds. Such comprehensive study concerning the contamination of the southern Baltic Sea waters by the residues of selected pharmaceuticals is therefore reported for the first time.

## 2. Materials and methods

### 2.1. Chemicals

Sulfapyridine and sulfamethazine were purchased from Serva (Weissensberg, Germany). All of the other pharmaceutical standards as well as acetonitrile (ACN) (LC–MS Chromasolv®) were obtained from Sigma–Aldrich (Steinheim, Germany). Methanol (MeOH) (HPLC – grade) was obtained from POCH S.A. (Gliwice, Poland) and hexane (HPLC – grade) from J.T. Baker (Deventer, Holland). Ammonium acetate ($NH_4Ac$), acetic acid (AcH) and sulfuric acid (all at analytical reagent grade) were purchased from Chempur (Piekary Śląskie, Poland). Deionized water was produced by the HYDROLAB System (Gdańsk, Poland).

Standard stock solutions (concentration of 500 μg $mL^{-1}$) were prepared by dissolving each compound in methanol, except the oxolinic acid, which was prepared in a mixture of 0.1 M NaOH:MeOH (10:90, v/v). Working solution (a mixture of each pharmaceutical in a concentration of 1 μg $mL^{-1}$) was prepared by diluting stock solutions in methanol. All of the standards were stored in the dark at −18 °C.

### 2.2. Sample collection and preservation

The water samples were collected in 2012 (using a stainless steel bathometer) during the cruises of the r/v Oceania (Institute of Oceanology, Polish Academy of Sciences) from various locations in the southern Baltic Sea along the Polish coastal zone. The details of the sampling sites are shown in Fig. 1 and Table 2. For optimizing the SPE procedure, seawater samples were collected from the Gulf of Gdańsk from the pier at Sopot.

The water samples were collected from the surface layer. From the station located at the Gdańsk Deep (SP 15a-d) samples were collected along the vertical profile from four different depths (surface waters, waters above the halocline, waters under the halocline and bottom waters). To analyze the horizontal profile river water and sea water samples were collected from the surface layer from locations at the Szczecin Lagoon/Pomeranian Bight (SP 7-11), the Słupia River/Słupia Mouth (SP 16a-b) and the Vistula River/Mouth of the Vistula (SP 17a-b).

Samples were filtered through 0.45 μm glass fiber filters (Macherey – Nagel) immediately after collection and then frozen in high density polyethylene (HDPE) bottles at −18 °C until analysis, which were performed within next four months. The salinity of the water samples was measured *in situ* using aHQ40d Dual-Input Digital Multi-Parameter Meter (Hach Lange). The amount of suspended matter in the seawater was determined by pouring a carefully measured volume of water through a filter (pre-weighed after ignition at 600 °C). The filter was weighted again after drying at temp. 60 °C. The amount of suspended matter was calculated as the gain in weight of a dry filter divided by the volume of filtered water (expressed in mg $L^{-1}$).

### 2.3. LC–ESI–MS/MS analysis

An Agilent 1200 Series LC system (Agilent Technologies Inc., Santa Clara, USA) was used. Analytes were separated on Gemini $C_{18}$ column (150 mm × 4.6 mm, 5 μm pore size) (Phenomenex Inc., Torrance, CA) at a temperature of 25 °C. Mobile phase A was a mixture of $H_2O$:ACN (90:10, v/v, 1 mM $NH_4Ac$/AcH, pH 3.5) and mobile phase B was 100% ACN. Elution began with 95% of mobile phase A, which was reduced to 36% within 32 min and then to 28% within the next 10 min. The injection volume was 50 μL.

The MS/MS system consisted of an HCT Ultra ion trap mass spectrometer (Brucker Daltonics, Bremen, Germany) equipped with an electrospray ionization source. For data acquisition, EsquireControl software was used. The source temperature was 300 °C. Nitrogen was employed as the nebulizer gas (30 psi) and the dry gas (10 L $min^{-1}$). In the ion trap, helium (99.999%) was used as the collision gas. All of the compounds were tested in both positive and negative ion modes. The parameters were optimized manually, separately for each compound. The best conditions for isolating the precursor ion was determined. After this, a full scan MS mode was used to record the product ions. For each compound, the fragmentation amplitude and isolation width were also optimized manually to increase the sensitivity and selectivity of the method and to select three of the most intensive and characteristic fragmentation ions for qualitative analysis. For quantitative analysis, the ion of the highest intensity was selected.

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