OCCURRENCE OF PHARMACEUTICAL RESIDUES IN THE COASTAL AREA OF THE SOUTHERN BALTIC SEA

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EXTENDED ABSTRACT

Little data about the occurrence of selected pharmaceuticals in the coastal area of the southern Baltic Sea are available. The Baltic catchment area is home to about 85 million people and various branches of drugs industry, intensive farming and animal husbandry are located in surrounding countries. The natural conditions of the Baltic Sea (a small depth and weak water exchange with the North Sea) might lead to exposure of this region for a constant supply and accumulation of selected compounds. For these reason the method has been developed for the trace analysis of 13 selected pharmaceuticals (sulfapyridine, sulfathiazole, sulfamethoxazole, sulfadimethoxine, sulfamethazine, trimethoprim, enrofloxacin, oxolinic acid, diclofenac, ibuprofen, ketoprofen, naproxen) in marine waters. The method employs solid-phase extraction with H₂O-Philic BAKERBOND Speedisk and liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) with electrospray ionization (ESI). Mass spectrometer was operated in multiple reaction monitoring mode (MRM).

The validation parameters of developed procedure showed good precision and accuracy with limits of quantification ranging from 0.5 and 10.0 ng/L. Recoveries were in the range of 61.31 to 98.26 %. It allows to identify and quantify selected pharmaceuticals in marine waters.

Samples of surface water were taken in 2012 and 2013 during r/v "Oceania" cruises from sampling stations located in the southern Baltic Sea along the polish coastal zone. The obtained results revealed that the residues of pharmaceuticals were present in this ecosystem. Eleven compounds were identified in the investigated samples at concentration level ng/L.

Keywords: environmental samples, pharmaceuticals, marine waters, Baltic Sea, solid-phase extraction, Speedisk, LC-MS/MS

1. INTRODUCTION

In recent years, the occurrence of pharmaceuticals in the aquatic environment has been increasingly raising concerns. Consequently, large numbers of reports have been published on traces of drugs detected in many different environmental samples, e.g., fresh-, and wastewaters, soils, sediments and biota [1-5]. However scientific information about presence of selected drugs in marine waters is still sparse.
At the onset of this investigation little data about the occurrence of pharmaceuticals in the coastal area of the southern Baltic Sea were available. The Baltic catchment area is home to about 85 million people and various branches of drugs industry, intensive farming and animal husbandry are located in surrounding countries. The Baltic is almost entirely land-locked and the water exchange is very limited. Its natural conditions might lead to exposure for a constant supply and accumulation of selected compounds [6].

Drugs selected for this investigation are one of the most frequently used. The study focused on non-steroidal anti-inflammatory drugs which, because of the availability of non-prescription, are consumed in large quantities [7]. The second group of selected pharmaceuticals are: quinolones, fluoroquinolones and trimethoprim. These drugs have been used for many years in human and veterinary medicine. The last group of drugs are sulfonamides which represent classes of synthetic antibiotics. They are one of the oldest group of veterinary chemotherapeutic agents. Sulfonamides have been widely used for more than 50 years (also in Poland), thanks to their low cost and their broad spectrum of activity in preventing or treating bacterial infections [8].

The main aim of this study was to determine target compounds (sulfapyridine, sulfathiazole, sulfamethoxazole, sulfadimethoxine, sulfamerazine, sulfamethazine, trimethoprim, enrofloxacin, oxolinic acid, diclofenac, ibuprofen, ketoprofen, naproxen) in seawater sampled in the coastal area of the southern Baltic Sea.

2. MATERIALS AND METHODS

Environmental water samples were collected from various locations in the southern Baltic Sea along the Polish coastal zone (Table 1) during research cruises performed aboard r/v Oceania (Institute of Oceanology, Polish Academy of Sciences).

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Site description</th>
<th>GPS coordinate</th>
</tr>
</thead>
<tbody>
<tr>
<td>SP 1</td>
<td>Gulf of Gdańsk – Output of Gdańsk-Wschód Wastewater Treatment Plant</td>
<td>54°21'567” N 18°54'500” E</td>
</tr>
<tr>
<td>SP 2</td>
<td>Mouth of the Vistula</td>
<td>54°23'098” N 18°57'354” E</td>
</tr>
<tr>
<td>SP 3</td>
<td>Gulf of Gdańsk</td>
<td>54°30'355” N 19°03'989” E</td>
</tr>
<tr>
<td>SP 4</td>
<td>Gulf of Gdańsk</td>
<td>54°22'941” N 19°03'989” E</td>
</tr>
<tr>
<td>SP 5</td>
<td>Szczecin Lagoon</td>
<td>53°41'382” N 14°30'278” E</td>
</tr>
<tr>
<td>SP 6</td>
<td>Pomeranian Bight</td>
<td>54°05'890” N 14°28'533” E</td>
</tr>
<tr>
<td>SP 7</td>
<td>Świnoujście- the port channel</td>
<td>53°57'885” N 14°13'871” E</td>
</tr>
<tr>
<td>SP 8</td>
<td>Puck Bay – Output of Gdynia-Dębogórze Wastewater Treatment Plant</td>
<td>54°36'807” N 18°33'665” E</td>
</tr>
<tr>
<td>SP 9</td>
<td>Gdańsk Deep</td>
<td>54°49'085” N 19°17'147” E</td>
</tr>
<tr>
<td>SP 10</td>
<td>Open sea (Słupia Mouth)</td>
<td>54°36'492” N 16°50'476” E</td>
</tr>
</tbody>
</table>

Analytes were separated on Gemini C18 column (150 mm x 4.6 mm, 5 μm pore size) (Phenomenex Inc., Torrance, CA) at a temperature of 25 °C. Mobile phase A was a mixture of H2O:ACN (90:10, v/v, 1mM NH4Ac/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 next 10 min. The flow rate was 0.3 mL/min, the injection volume was 50 μL and the analytical wavelength was 270 nm.
The LC flow was directly introduced into the MS. The MS/MS system consisted of an HCT Ultra ion trap mass spectrometer (Bruker Daltonics, Bremen, Germany) equipped with an electrospray ionization source. The source temperature was 300 °C. Nitrogen was employed as the nebulizer gas (30 psi) and the dry gas (10 L/min). As the collision gas in the ion trap helium (99.999 %) was used. For qualitative analysis mass spectrometer was operated in multiple reaction monitoring mode (MRM). A precursor ion and two product ions were selected for every compound. During the run the system was in positive ESI-mode (for sulfonamides, trimethoprim, enrofloxacin and oxolinic acid) and in negative ESI-mode (for non-steroidal anti-inflammatories). For quantitative analysis the ion of the highest intensity was selected.

The solid phase extractions were performed with the use of BAKERBOND Speedisks (H₂O-Phlic DVB) of J.T. Baker (Heidelberg, Germany). Samples pH, prior to the extraction, was adjusted to 3.5 with concentrated H₂SO₄. The SPE disks were sequentially preconditioned with 40 mL of MeOH and 40 mL of deionized water. Each of 1.5 L sample was then loaded onto the disk, after that they were rinsed with 20 mL of MeOH:H₂O (2:98, v:v) and dried under the vacuum by 20 minutes. The SPE disks were secondly washed with 30 mL of hexane and dried by 20 minutes. The analytes were eluted with 20 mL of MeOH. After elution, extract was evaporated to dryness under a gentle stream of nitrogen and reconstituted in 1.5 mL of mobile phase A. The sample was centrifuged and analyzed by LC–MS/MS.

3. RESULTS

The validation parameters of the presented procedure showed good precision and accuracy with limits of quantification ranging from 0.5 and 10.0 ng/L. Recoveries were in the range of 61.31 to 98.26 %. Thus the method enabled to identify and quantify certain pharmaceuticals present in environmental samples at low concentrations.

The obtained results revealed that the residues of pharmaceuticals were present in this ecosystem – all of the investigated samples contained drug residues. As expected, most of them were found in the sample collected close to the output of the Gdańsk – Wschód Wastewater Treatment Plant (SP 1), the Output of Gdynia – Dębogórze Wastewater Treatment Plant (SP 8) and collected from the Słupia Mouth (SP 10).

Eleven compounds were identified in the investigated samples at concentration level ng/L. The compound most frequently detected was trimethoprim, which was found in all samples. The maximum concentration was determined for ketoprofen (135.0 ± 10.9 ng/L). In none of the samples oxolinic acid and naproxen were determined.

4. CONCLUSIONS

This manuscript discusses the results of the study on the presence of selected pharmaceuticals (sulfapyridine, sulfathiazole, sulfamethoxazole, sulfadimethoxine, sulfamerazine, sulfamethazine, trimethoprim, enrofloxacin, oxolinic acid, diclofenac, ibuprofen, ketoprofen, naproxen) in the seawater samples. The proposed method was used to analyse environmental samples taken from the coastal area of the southern Baltic Sea. The results revealed that several compounds are presented at concentration level ng/L. The tested pharmaceuticals were determined for the first time in this ecosystem. However, to conclude about the possible effects of pharmaceutical residues in the Baltic Sea, larger data sets should be taken into account. The analyses of the environmental samples will be continue.
ACKNOWLEDGEMENTS

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