Monitoring of selected pharmaceuticals in surface waters of the Vltava River Basin

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Keywords: Vltava River Basin, OTC medicines, antibiotics, anticolvulsants, contrast agents, LC-MS/MS

1 Introduction

The Framework Monitoring Programme (MoE 2007), developed in line with EU directives (EU 2000, 2008) by the Ministry of the Environment and Ministry of the Agriculture of the Czech Republic in cooperation with the Czech Hydrometeorological Institute (CHMI), defines the lists of chemical and microbiological indicators for the assessment of chemical and ecological status of surface waters. In the last 5 to 10 years, the list of monitored active substances of pesticides used in the Czech Republic for plant protection products, wood preservatives etc. is been expanding along with the rapid development of instrumental analytical separation methods with mass detection. To respond to the positive findings of substances belonging to the specific group referred to as PPCPs (Pharmaceuticals and Personal Care Products) in environment, new multi-analytical methods have also been developed, allowing to identify these substances, including, e.g., synthetic musk compounds, complex-forming compounds, hormones, active ingredients of pharmaceuticals. These substances are discharged into aquatic ecosystems via municipal sewage treatment plants (STPs) effluents. An efficient technology of PPCPs degradation in the process of waste water treatment is yet to be developed, PPCPs are not monitored in STPs effluents, so consequently, mass balance cannot be calculated. Health risks and adverse impacts on the biological components in the river ecosystem are the subject of many scientific and technical publications around the world. The situation is complicated by the diversity of pollutants and their biological activities, which enable the formation of degradation products whose impacts and risks may differ substantially from those of the initial substances (Fuksa et al. 2010b). This paper is focused on the monitoring of pharmaceuticals belonging to OTC (Over-The-Counter) drugs – ibuprofen and diclofenac from the group of NSAIDs (non-steroidal anti-inflammatory drugs) and carbamazepine (anticonvulsants). In addition, erythromycin, sulfamethoxazole (antibiotics) and iopromide and iopamidol (contrast agents) have also been monitored since January 2010. These pharmaceuticals are registered by the State Institute for Drug Control and some of them are high on the Czech theoretical drug consumption list (www.sukl.cz, 2009). Drug determination at concentrations reaching the ambient pollution levels is relatively new to the chemical analysis of water. Expansion of liquid chromatography with tandem mass spectroscopy (LC-MS/MS) provided conditions for developing the methods of determination of drug levels in water. It is also important in this context that extremely sensitive mass detectors have been developed and are currently available at a reasonable price, affordable even to routine water management laboratories. Although currently drugs are not comprised in the Framework Monitoring Programme of the Czech Republic and in current national legislative documents, the above drugs are being monitored for already the second year at specific international sampling sites under the Elbe Monitoring Programme (IKSE-MKOL 2010). Povodí Vltavy, státní podnik (The Vltava Valley Authority) is responsible for the management of watercourses and for evaluation of surface water quality, and therefore it has been decided that this new group of drugs should be included in Povodí's programme of surface water operational monitoring to obtain basic information on pollution at selected sampling sites. The aim of this study was, first, to determine which of the monitored rivers were the most loaded with drugs, and then to try and identify the causes of high concentrations and, finally, to define the general nature of pollution; generally, the objective is to identify or at least suggest other areas with high drug concentrations.

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2 Analytical methods

A new analytical method was developed for the analysis of drugs in surface waters (Seitz et al. 2006; Chin-Kai 2006; Borton et al. 2007; EPA 2007; Kule et al. 2009). Liquid chromatography was used for separation, and tandem mass spectrometry was used for detection. In choosing the sample preparation, it was necessary to take into account the physical and chemical properties of analytes, especially their higher polarity and lower stability. Sample preservation from the time of sampling to the time of analysis (freezing) was therefore emphasised in the methodology. The technique uses a unique principle of detection, based on the determination of the characteristic MRM transmission between the precursor and product ion, and therefore the samples did not need to be cleaned. For liquid chromatographic analysis, a centrifuged water sample was injected directly, without any organic solvent extraction and without SPE or similar techniques. To achieve the required limit of determination, it was necessary to increase the sample injection volume to 1 ml. A combination of the standard addition method and internal standard method with isotope-marked standards is used to gain information on the elimination matrix interference and to ensure quality control. The limits of quantification, MRM transmissions and the RSD repeatability of the LC-MS/MS method with direct injection of large volumes of sample are given in Table 1.

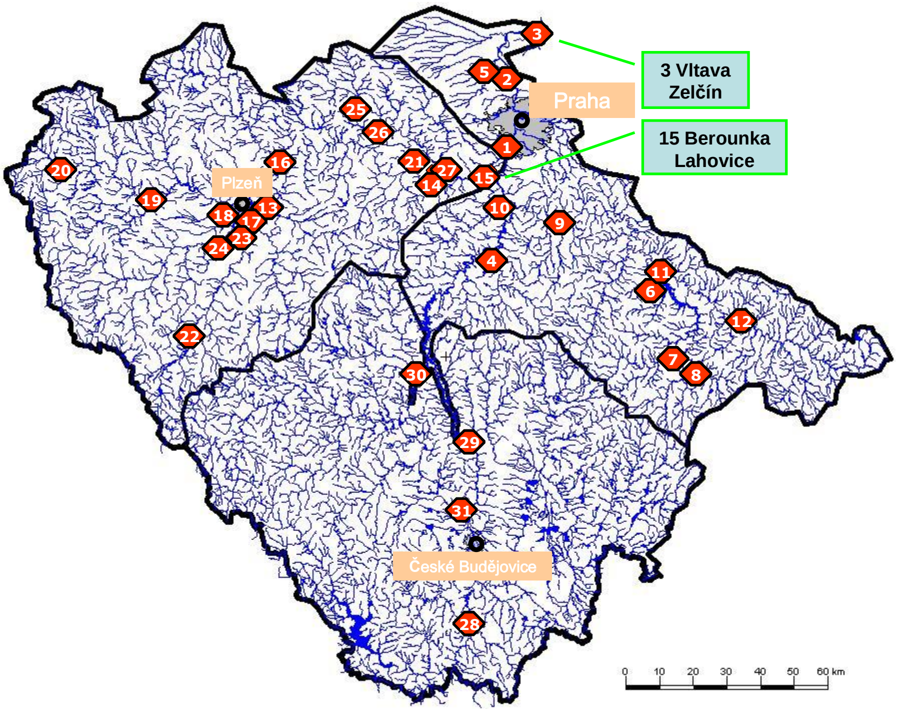
Table 1. *Quantitative and qualitative MRM transmissions and statistical parameters: RSD of repeatability for a concentration of 0.05 µg/l and analytical limit of quantification (LOQ).*

Name	MRM QUANT.	MRM QUAL.	RSD (%)	LOQ (µg/L)
Ibuprofen	205.2 → 161.0		6.3	0.05
Diclofenac	294.1 → 250.1	294.1 → 213.9	6.4	0.05
Carbamazepin	237.1 → 194.0	237.1 → 192.1	5.3	0.02
Iopromide	792.0 → 573.0	792.0 → 558.8	22.9	0.1
Iopamidol	775.7 → 126.6		15[1]	0.1
Sulfamethoxazole	254.1 → 155.9	254.1 → 91.9	7.2	0.02
Erythromycin	734.4 → 158.1	734.4 → 576.4	12.2	0.02

[1] A qualified estimate

3 Preparation of monitoring

Sampling sites were selected with regard to the possible sources of waste water containing drugs: first, in the international profiles (Vltava at Zelčín and Berounka at Lahovice), followed by the final sites of backbone flows, where pollution is to be recorded from a larger area, such as a small river basin, which typically corresponds to an area of several water bodies.



1 Vltava Podolí
2 Vltava Libčice
3 Vltava Zelčín
4 Mastník Radíč
5 Zákolanský str. Kralupy
6 Želivka ÚV Hulice
7 Želivka Poříčí
8 Bělá Pelhřimov pod
9 Benešovský str. Benešov pod
10 Sázava Pikovice
11 Sázava Zruč nad Sázavou
12 Sázava Chlístov
13 Berounka Bukovec
14 Berounka Srbsko
15 Berounka Praha Lahovice
16 Střela Borek
17 Úslava Plzeň Doubravka
18 Mže Plzeň Roudná
19 Mže Stříbro pod
20 Mže Oldřichov
21 Litavka Beroun
22 Úhlava Svrčovec
23 Úhlava Plzeň Doudlevce
24 Radbuza Dobřany pod
25 Rakovnický str. Dolní Chlum
26 Rakovnický str. Křivoklát
27 Loděnice Hostim
28 Malše Pořešín
29 Lužnice Bechyně
30 Otava Topělec
31 Vltava Hluboká nad Vltavou

Figure 1. *Map of the Vltava river basin with a sampling sites list. Vltava at Zelčín and Berounka at Lahovice are the sites of the IKSE-MKOL international monitoring programme.*

Simultaneously, monitoring was initiated at sites with a high population density where surface waters are affected by large amounts of treated municipal waste water discharged into a river, and also in areas where surface water is used as a source of drinking water. A total of 31 sites, whose locations are indicated in Figure 1, were selected for 2009. Sampling was carried out on a monthly basis. The initial set of 12 data items was thus generated, providing a first indication of pollution in the region.

4 Results

Figure 2 shows the average annual concentrations of ibuprofen, diclofenac and carbamazepine. Extremely high concentrations not recorded repeatedly, which did not reflect the standard conditions in the river, were excluded during data processing. As to concentrations below the limit of quantification, half the LOQ was used in the calculation (Nesměrák 2009). Table 2 shows the hydrological and population data from the sites where increased concentrations of drugs were regularly recorded. Monthly concentrations are shown in Figures 5-8.

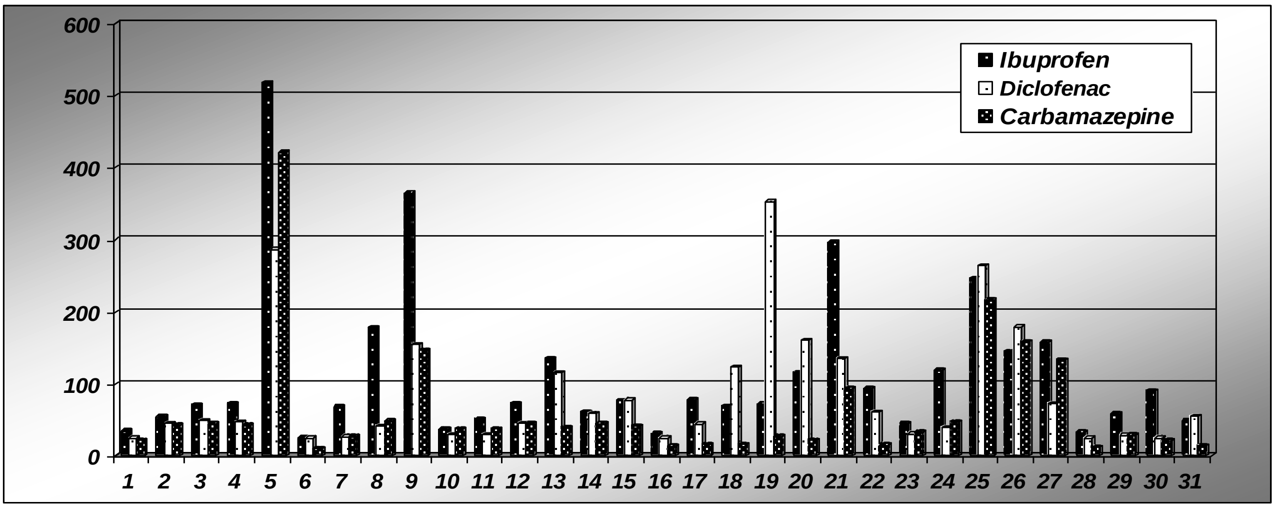
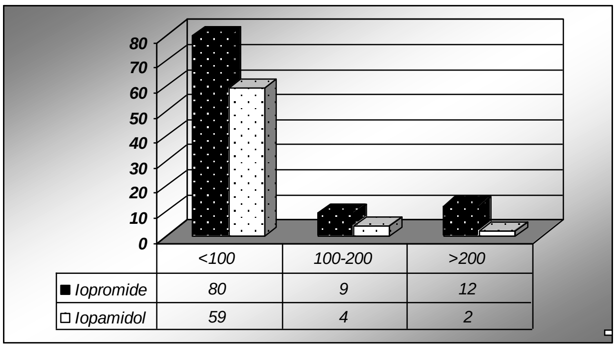
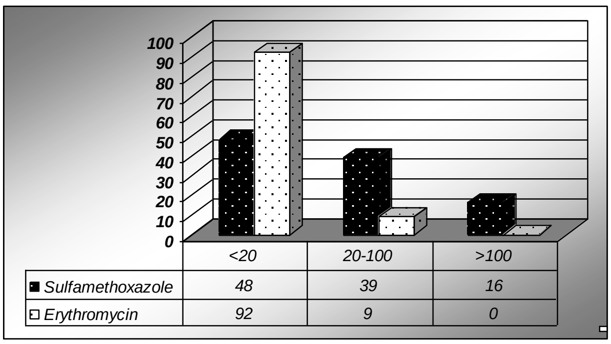


Figure 2. *Average concentrations (ng/l) of ibuprofen, diclofenac and carbamazepine (2009). Numbers on x axis identify the names of sampling sites (see sampling sites list in Figure 1).*

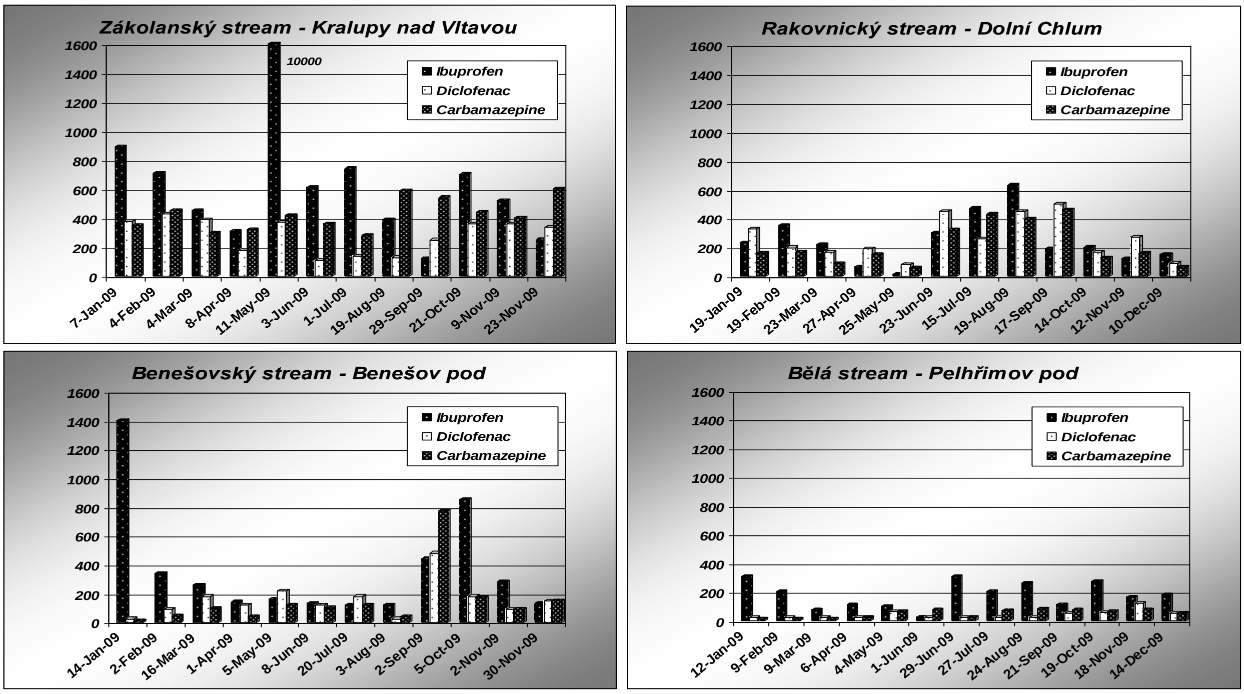
Table 2. *Hydrological and population data for the river basins with the highest drug levels.*

Name	Average discharge [m^3/s]	length [km]	drainage area [km^2]	population estimate
Rakovnický stream (Rakovník)	0.68	48.5	302.2	32,000
Zákolanský stream (Kralupy n/Vlt.)	0.63	28.2	265.6	107,000
Benešovský stream (Benešov)	0.33	17.2	80.7	17,000
Bělá stream (Pelhřimov)	0.87	22.7	94.2	20,000





Figures 3-4. *Distribution of values for iopromide, iopamidol, sulfamethoxazole and erythromycin (ng/l). Data was collected in November and December 2009.*



Figures 5-8. *Monthly concentrations (ng/l) of ibuprofen, diclofenac and carbamazepine. The sites with the highest concentrations in the Vltava River basin are small rivers (flow <1 m^3/s) to which a large quantity of wastewater is discharged from large cities (15-100 thousand inhabitants).*

Data for iopromide, iopamidol, sulfamethoxazole and erythromycin obtained in November and December 2009 represent only a small file, not allowing to calculate annual average concentrations. Distribution graphs for drug concentrations at all sampling sites were therefore created from all values (Figures 3-4).

5 Discussion

Figures 5-8 show rivers with a high frequency of concentrations above the limit of quantification for most of the drugs under review, often reaching hundreds of ng/l. What these sites have in common is a medium-sized city with a population of 15-100 thousand, whose municipal waste waters are discharged into a small watercourse with a flow rate of less than 1 m^3/s. Hence, an area where high concentrations of drugs are likely to occur can generally be characterised as “a large town on a small river”. Extreme drug concentrations as a rule disappear at the confluence of such a small stream with a large river. Therefore, the health risks caused by the toxic mixtures of drugs and their adverse impacts on the biological components of flowing surface waters can be expected to occur specifically in small watercourses. What can also be considered important is the fact that the ongoing monitoring is limited to only a few analytes, although as many as about 3000 active substances of medicinal products are known to be registered in the Czech Republic. Those used most widely include contraceptive pills, antibiotics, OTC drugs, etc. (Fuksa et al. 2010b). Summarising the following facts, we can obtain a detailed overview of the issues of drugs escaping into the environment, their impacts and the health risks to the ecosystem of surface waters in the Czech Republic:

- There is no balance monitoring of drugs in surface waters to provide information about the total amount of these substances in the aquatic environment.
- Research projects concerning the overall balance of drugs in waste waters (at inflow to treatment plants, in the treatment process, at the discharge from treatment plants) led to the conclusion that efficient removal is needed. The main reason is that with some of the drugs (carbamazepine) it will be impossible to find a consistent balance between the theoretical consumption and supply (Fuksa et al. 2010a).
- Technologies to remove pharmaceuticals from waste water are explored only marginally and specific applications are rare (WWTP Modřice: Vávrová et al. 2009; Jedličková at al. 2010).
- The range of the analytes being monitored is very narrow; for example, it does not include the active substances of hormonal contraceptives.

- Analysis of the impact of drugs on the biological components of rivers (fish, macrozoobenthos) in critical areas of the Czech Republic is not systematically documented.

To meet these challenges, state authorities (ministries, the Czech Hydrometeorological Institute, river valley authoritires), need to cooperate with the specialized departments of universities, with the T.G.Masaryk Water Research Institute p.r.i. Prague, etc. Sufficient funding must be provided (operational monitoring, research grants, etc.) in addition to the technical and scientific solutions.

6 Conclusions

Operational monitoring of selected drugs showed that these substances are present in surface waters at measurable concentrations, often in hundreds of ng/l. Municipal sewage treatment plants are the sources of the drugs. The concentrations are particularly high where the waste water dilution ratio is low (a big city on a small river). It is time now to fill the gaps in monitoring programs and research on ecological impact of the PPCPs.

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www.sukl.cz (2009) keywords Carbamazepine, Diclofenac, Ibuprofen, Iopromide, Iopamidol, Sulfamethoxazole, Erythromycin.