**IOSR Journal of Engineering (IOSRJEN)** **www.iosrjen.org**
ISSN (e): 2250-3021, ISSN (p): 2278-8719
Vol. 05, Issue 10 (October. 2015), ||V2|| PP 35-45

# New sampling method for routine monitoring of selected pharmaceuticals in surface water

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***Abstract:*** The surface waters contain trace levels of pharmaceuticals therefore powerful analytical methods and/or extraction pre-treatment of samples are necessary. But these methods are very expensive and their utilization is therefore limited especially for nonprofit ecological organizations. The aim of this study was to develop a cheap sampling method for routine monitoring of Ibuprofen, Cyproteron acetate, Desogestrel, Ethynilestradiol and Gestodene contents in aquatic environment. The sampling method was developed for sample collection and for concentration enrichment. Proposed sampling method is based on utilization of bags filled with crushed dried *Reynoutria Bohemica* which were tested by sorption and desorption experiments with model aquatic samples. The sorption capacities were determined from 37.5 to 77.5 mg/g. The optimum desorption conditions were determined (desorption efficiencies from 60.2 to 94.3%). Two UHPLC/MS methods were developed and validated for determination of pharmaceuticals concentrations. Obtained validation parameters enable utilization of these methods for analysis of trace levels of above-mentioned pharmaceuticals. Developed sampling method was applied to monitoring of studied pharmaceuticals contents in surface waters of Moravian-Silesian Region (Czech Republic). The contents of above-mentioned pharmaceuticals in studied waters were established in tens ng/g/24h. It can be stated that proposed method is suitable for routine sampling of surface waters**.**

***Keywords:*** *Liquid chromatography; Mass spectrometry; Pharmaceuticals; Sampling, Water contamination*

## I. INTRODUCTION

One of the most relevant topics in the environmental analytical chemistry is water quality which is primarily affected by contaminants – for example by heavy metals [1]. Pharmaceuticals (substances for prevention and medication of diseases) are also classified as significant pollutants of the environment [2-4]. Presence of the pharmaceuticals in the nature is related to their increasing consumption. Human metabolism is an imperfect process so the amount of non-metabolized pharmaceuticals excreted into the urine increases. This excess together with incorrect disposal of expired pharmaceuticals have an impact on the subsequent increase of non-metabolized pharmaceuticals content in wastewater. The imperfections in wastewater treatment plants then lead to contamination of the surface water [2-4]. Various kinds of pharmaceuticals such as antibiotics [5,6], antidepressants [7, 8], diuretics [9], hypnotics [10, 11], sedatives [10, 11], opiates [3, 11], nonsteroid anti-inflammatory drugs [2, 12-14] or hormonal contraception [15-17] were found in the surface water around the world. The surface water could be then a source of contamination of drinking water [18], groundwater [18], soil [19] and agricultural crops [20, 21] and a source of the problems of aquatic organisms [22, 23].

The characterization of contamination by pharmaceuticals requires powerful analytical method which is able to quantify these pollutants at very low concentrations (few ng/L). Therefore, gas and liquid chromatography in connection with mass spectrometry have been mainly used for analysis of pharmaceuticals in natural waters [8, 17, 24, 25]. However, it is unsafe to make direct injection of natural water samples into these instruments because it may cause their contamination or failure. Thus, some pre-treatment of the sample is very useful before instrumental analysis. The sample pre-treatment is mostly performed by solid phase extraction (SPE) [10, 16, 26, 27] which is very effective. Unfortunately, SPE is expensive for many organizations.

Also the sample collection is not a simple process. Generally, even the best sample pre-treatment or instrumental method cannot eliminate errors caused by inexpert sample collection. Sampling of the surface water into a suitable container followed by the above-mentioned SPE [28, 29] is nowadays the most widely used sampling strategy. However, it requires the utilization of a suitable container and a sampler for the sampling. In addition, it is necessary to follow strictly the prescribed procedures for sampling depending on what is the subject of monitoring (for example procedures for monitoring of the microorganism content are different from those for the monitoring of heavy metals content) [28, 29]. An application of passive samplers [30] is an alternative way for the simplification of sampling process. However, this simplification requires higher acquisition cost of the passive samplers.

The main goal of this study is to develop the new sampling method based on sorption on the plant material (*Reynoutria Bohemica*) for routine and simple analysis of Ibuprofen (IBU), Ethynilestradiol (EES), Cyproteron acetate (CA), Desogestrel (DES) and Gestodene (GES) in surface waters. The selection of the tested pharmaceuticals was based on the following facts: Ibuprofen is one of the most popular painkillers in the Czech Republic and the selected 4 hormonal contraceptives (Ethynilestradiol, Cyproteron acetate, Desogestrel and Gestodene) are found as the most sold contraceptive products in the Czech Republic. The proposed sampling method combines sample collection and pre-treatment in one step which reduces costs and also possibility to make mistakes during sampling. Also final instrumental analysis methods using UHPLC/MS were created and validated so the complete sampling strategy is created.

## II. MATERIALS AND METHODS

### 2.1 Materials and chemicals

The bags were prepared from white non-woven textile Startex 17 (NOHEL GARDEN a.s., Czech Republic). This textile is normally used for covering of crops (protection against pests and the inclement weather – for example frost, hail) on the garden.

Fresh leaves of *Reynoutria Bohemica* were obtained on the banks around Odra river (see point OD in part 2.6). The leaves were dried on the air and crushed by the laboratory mill IKA M20 (IKA® -Werke GmbH & Co. KG, Germany).

Acetonitrile (ACN), methanol (MeOH) and water (W) (MS purity, Merck KGaA, Germany) were used for preparing of extraction solutions and mobile phases for UHPLC/MS, respectively.

Pharmaceuticals stock solutions were prepared with deionized water produced by reverse osmosis (Aqual 25, AQUAL s.r.o., Czech Republic).

Standards of IBU, EES, CA, DES and GES (Dr. Ehrenstorfer GmbH, Germany) were used for preparation of stock solutions for development and validation of both analytical (UHPLC/MS) methods.

The Ibalgin 400 tablets (Zentiva, k.s., Czech Republic) were used as a source of Ibuprofen for sorption tests of bag method. The tablet was dissolved in 1 L of deionized water and the Ibuprofen concentration in the sorption solution was verified by UHPLC/MS. The hormonal contraception stock solutions prepared for validation of UHPLC/MS method were used after dilution also for sorption tests. Concentrations of sorption solutions were 400 mg/L (IBU) and 500 mg/L (hormonal contraceptives).

### 2.2 Development and validation of analytical method

Two analytical methods were developed using UHPLC/MS system Ultimate 3000/micrOTOF-QII (supplier Bruker s.r.o., Czech Republic) equipped with column Kinetex 5μ C18 100A (Phenomenex, USA). One method was developed for IBU determination and second method was developed for hormonal contraception determination.

Flow injection analysis (FIA) was used for the determination of detection masses and ionization mode at MS. Moreover, optimization of liquid chromatograph and mass spectrometer parameters was performed to obtain maximal response (sensitivity) from the system. Parameters changed during optimization are listed in the Table 1.

The developed methods were evaluated by validation parameters [11, 31-33]. Validation was performed with solutions in the concentration range from 0.1 ng/L to 100 ng/L prepared from the above-mentioned standards. The limits of detection (LOD) and quantification (LOQ) were determined through a diminishing of the concentration of the prepared sample solutions. The response for the specific concentration was always compared with the response of the blank and afterwards signal:noise (S:N) ratio was determined. LOD and LOQ were defined as the concentration at which the S:N ratio was minimally 3:1 (LOD) and 10:1 (LOQ) [11, 31-33]. The linearity was evaluated on the basis of linearity determination coefficients ($R^2$) which were obtained by measurement of seven-point calibration curve (concentrations 1, 5, 10, 25, 50, 75 and 100 ng/L) with five repetitions. The repeatability and reproducibility at three concentration levels (1, 10, 100 ng/L) were determined. The repeatability at each concentration was determined from 20 measurements performed during one day. The reproducibility was determined from 5 measurements performed during five consecutive days. The determination of precision and accuracy were performed with the results of analyses obtained by repeatability determination. Precision was defined as a deviation of 95% confidence interval from the mean value of this interval. Accuracy was determined as the deviation of the mean value of 95% confidence interval from the value held as true [11, 31-33].

### 2.3 Sampling accessory

The rectangle pieces with dimensions 16x8 cm were cut out from white non-woven textile. These pieces have been folded in a half and welded on two lateral sides by foil welding machine FS40 (Petra Electric, Germany). The prepared bags were filled with 250 mg of crushed dried leaves of *Reynoutria Bohemica* and then they were sealed on the last side and provided with eyelet (for future application in a real environment) (Fig. 1).

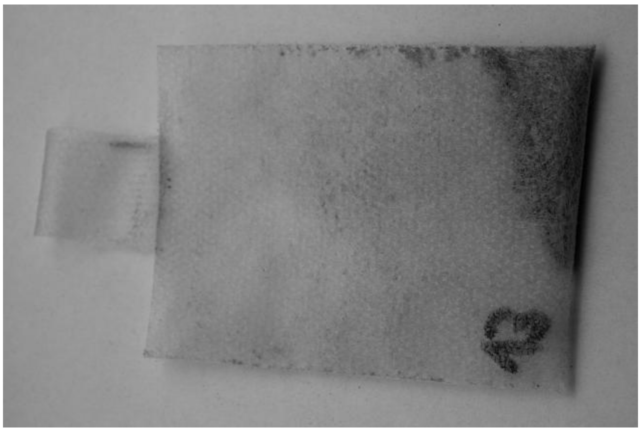

Fig. 1. The final pattern of bag with crushed leaves of *Reynoutria bohemica*.

Simple sampler was developed for the surface water sampling (Fig. 2). Sampler contains 3 mounts for installing sampling bags, float and weight as an anchor. Developed sampler allows to take 3 parallel determinations from each sampling side.

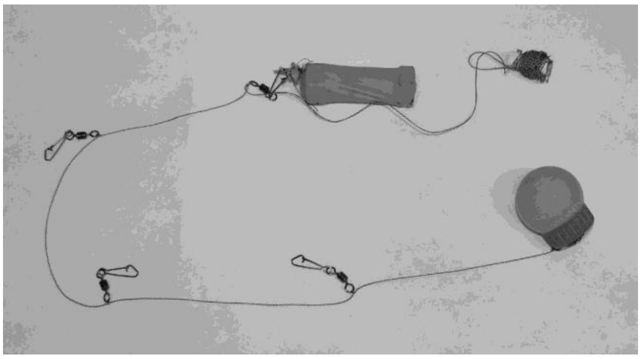

Fig. 2. The **s**ampler developed for sampling of real water samples.

### 2.4 Infrared spectroscopy of used sorbent

Sorbent used in this work (*Reynoutria Bohemica*) is natural plant material. The infrared spectra of the sorbent were measured for estimation of homogeneity of the material. Spectra were measured on the Nicolet 6700 (Thermo Scientific, USA) instrument with KBr beamsplitter and DTSG/KBr detector. The attenuated total reflectance (ATR) technique with single bounce diamond crystal was used. Spectra were collected in the range 4000 – 400 $cm^{-1}$, with resolution 4 $cm^{-1}$. Number of scans collected during spectrum accumulation was 256. Three samples were taken from the bulk of crushed sorbent and each sample was measured 5 times. Spectra of each sample were averaged and baseline correction was performed.

### 2.5 Sorption and desorption properties of sampling bags

The maximum possible adsorbed amounts (sorption capacities) of pharmaceuticals were determined by batch method for bags with adsorbent. Filled bag was inserted into Erlenmayer flask with 50 ml of pharmaceutical solution with concentration 400 mg/L of IBU and 500 mg/L of hormonal contraceptives. Each pharmaceutical was tested individually. The volume of 50 ml was chosen with regard to laboratory practice which is currently used for the determination of the sorption isotherms [34-36]. Smaller volumes (for example 10 ml) are also commonly used [37]. The sorption system was shaken for 24 hours at the laboratory shaker Unimax 1010 (Heidolph Instruments GmbH & Co.KG, Schwabach, Germany) at 250 rpm, at temperature approximately 20°C and at atmospheric pressure. The bag was removed after 24 hours, solution was filtered through the syringe filter (PTFE, 0.2 μm, Whatman Inc., USA) and it was subsequently analyzed by developed UHPLC/MS methods.

The desorption efficiencies were determined by 6 different types of extraction agents – water (W), acetonitrile (ACN), methanol (MeOH) and mixtures of these solvents in v:v:v ratios (ACN:W:MeOH) 50:50:0, 50:0:50, and 0:50:50. The same volume (50 mL) of extraction agent and the same parameters (time, shaking, temperature and pressure) as by the adsorption experiment were applied. The extracts were analyzed by developed UHPLC/MS methods after filtering through the above-mentioned syringe filter.

Adsorption and desorption experiments were carried out in five parallel determinations for each pharmaceutical and desorption agent.

The same procedures were carried out with an empty bag to eliminate the errors resulting from adsorption and desorption of used pharmaceuticals on bag material.

### 2.6 Sampling method testing in the natural environment

Developed sampling method was finally tested for water contamination monitoring by IBU, EES, CA, DES and GES of surface water in Moravian-Silesian Region. The testing was performed in June 2013 on five sampling places – three lakes (Kališok - KA, Antošovice - AN and Hlučín - HL) and two rivers (Ostravice - OS and Odra - OD) (see Fig. 3 [38] for location). Both rivers belong to the most important watercourses of Moravian-Silesian region which drain away a water into Poland. Selected three lakes belong to the most important water areas that are used for recreation purposes (swimming or fishing).

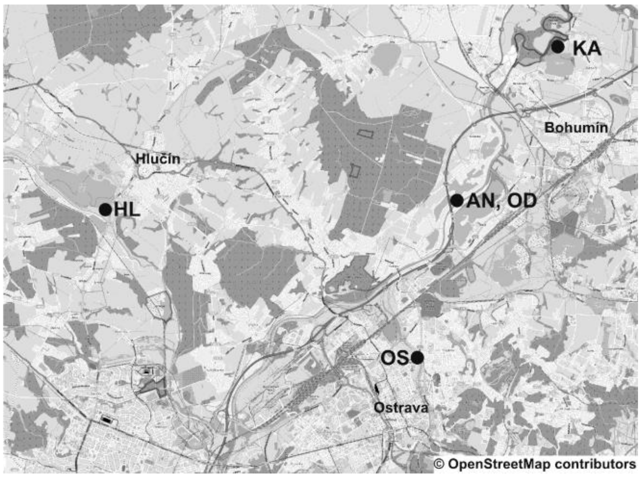

Fig. 3. Sampling locations in Moravian – Silesian Region, Czech Republic (OS – Ostravice river, OD – Odra river, AN – lake Antošovice, KA – lake Kališok, HL – lake Hlučín). The cartography in the map tiles and documentation are licensed under the Creative Commons Attribution-ShareAlike 2.0 license (CC BY-SA). The maps are available under the Open Database License on address - http://www.openstreetmap.org/#map=12/49.8718/18.2208 [38].

Sampling was performed in a three-day cycle. The sampling bags were thrown up on sampler into the water in selected areas at the first day. The exact sampling locations were chosen according to safety of used samplers (theft of samplers was registered) and to avoid the interference (for example to fishermen). The bags were removed after 24 hours of exposition (second day) and they were inserted in the Erlenmayer flasks with 50 mL of extraction agent. Prepared extracts were filtered through the syringe filter (PTFE, 0.2 μm, Whatman Inc., USA) into vials after 24 hours of extraction and then filtrates were analyzed by developed UHPLC/MS methods.

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## III. RESULTS AND DISCUSION

### 3.1 Development and validation of analytical methods

The determinations of ionization mode and detection masses at MS instrument were performed by flow injection analysis (FIA). The negative electrospray ionization with detection mass 205.1 Da was determined as the best for the Ibuprofen analysis. On the contrary, positive electrospray ionization was preferable for determination of hormonal contraceptives with following detection masses: Ethynilestradiol – 321.1 Da, Cyproteron acetate – 417.2 Da, Desogestrel – 368.2 Da and Gestodene – 349.2 Da. Two UHPLC/MS methods were developed due to difference in the ionization mode for IBU and contraceptives determination. One method was developed for IBU determination and second method was developed for hormonal contraceptives determination.

Subsequently, the optimizations of LC and MS parameters were performed. Final optimized LC and MS parameters for both isocratic methods are shown in Table 1. Commonly used methods for Ibuprofen and hormonal contraception determination are characterized by long time of analysis (10 minutes and more) [2, 8, 12, 16, 17]. The main goal of our optimized UHPLC/MS methods is a shortening of analysis run time down to

3.5 minutes for both Ibuprofen and hormonal contraception (Fig. 4 and 5). Shorter analysis time means economization of analysis and also leads to green chemistry due to lower consumption of chemicals.

Table 1 Characteristics of developed isocratic methods (IBU – method for Ibuprofen determination; HC – method for determination of hormonal contraception; ACN – Acetonitrile; MeOH – Methanol; W - Water)

| Part | Parameter | IBU | HC |
|---|---|---|---|
| LC | Mobile phase | 7:2:1 (v:v:v) ACN:MeOH:W | 5:4:1 (v:v) ACN:MeOH:W |
| | Mobile phase flow | 0.2 mL/min | 0.15 mL/min |
| | Column temperature | 20°C | 20°C |
| | Injection | 100 μL | 100 μL |
| MS | Ionization | ESI- | ESI+ |
| | Capillary voltage | 2900 V | - 4500 V |
| | Nebulizer gas pressure | 2 bar | 1.5 barr |
| | Drying gas flow | 10 L/min | 7 L/min |
| | Drying gas temperature | 200°C | 220°C |

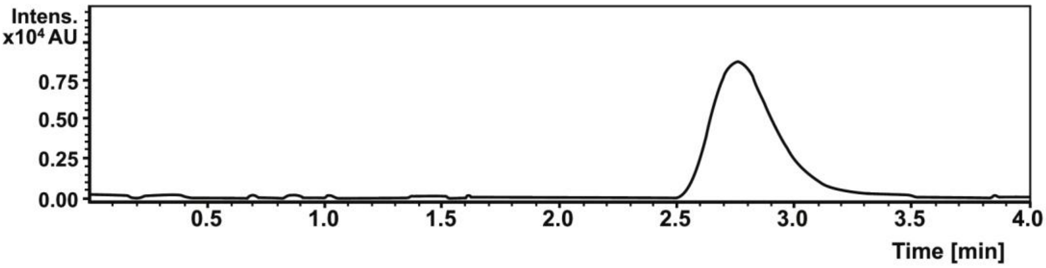

Fig. 4. Extracted mass chromatogram of Ibuprofen analysis (ESI-, m/z 205.1 Da) with concentration 50 ng/L.

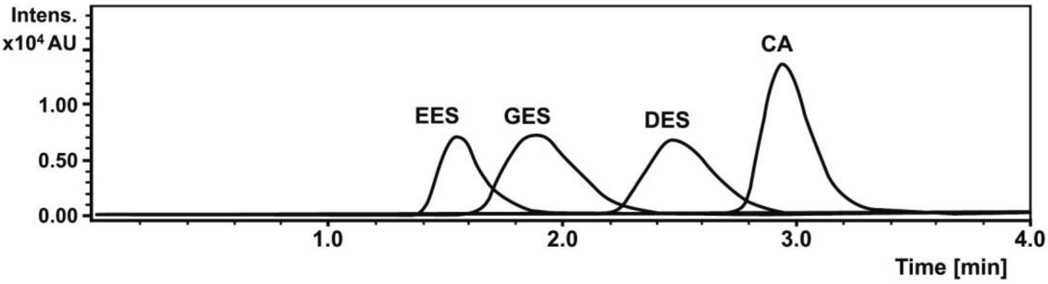

Fig. 5. Extracted mass chromatograms of hormonal contraception mixture analysis (ESI+, EES – Ethynilestradiol (m/z 321.1 Da), GES – Gestodene (m/z 349.2 Da), DES – Desogestrel (m/z 368.2 Da), CA – Cyproteron acetate (m/z 417.2 Da) with concentration of each substance 50 ng/L.

Two optimized methods were subsequently validated – the limits of detection and quantification, determination coefficients of linearity, repeatability, reproducibility, precision and accuracy were determined. Determined validation parameters of both methods are shown in Table 2.

Table 2 Validation parameters of developed methods (IBU – method for Ibuprofen determination; HC – method for determination of hormonal contraception)

| Parameter | IBU | HC |
|---|---|---|
| Limit of detection | 0.1 ng/L | 0.3 ng/L |
| Limit of quantification | 0.5 ng/L | 1 ng/L |
| Determination coefficients of linearity | 0.990 – 0.994 | 0.990 – 0.994 |
| Repeatability (maximal deviation) | 6.36% | 7.25% |
| Reproducibility (maximal deviation) | 9.46% | 9.92% |
| Precision (maximal deviation) | 2.85% | 4.21% |
| Accuracy (maximal deviation) | 1.37% | 2.20% |

Racamonde [10], Fedorova [22] or Patrolleco [16] detected pharmaceuticals in the surface waters in the concentration range from tens to hundreds ng/L. The obtained limits of detection (0.1 ng/L for IBU and 0.3 ng/L for hormonal contraceptives) and especially limits of quantification (0.5 ng/L for IBU and 1 ng/L for hormonal contraceptives) indicate that both our developed analytical methods are suitable for direct analysis of aquatic samples containing the selected pharmaceuticals. The linearities of both analytical methods were evaluated by the linearity determination coefficients which are better than the limit 0.990 stated in literature [11, 31-33]. It can be stated that dependence of signal on the concentration is linear in the range from LOQ to 100 ng/L. The validation continued with determination of repeatability and reproducibility at three concentration levels 1, 10, 100 ng/L. The deviations of repeatability (6.36% and 7.25%) and reproducibility (9.46% and 9.92%) should not exceed 10% [11, 31-33]. The obtained parameters are better than results stated in literature. Similarly, determination of precision (deviations 2.85% and 4.21%) and accuracy (deviations 1.37% and 2.20%) were carried out. Both analytical methods are evidently acceptable according to values of precision and accuracy stated in the published studies [11, 31-33].

The values of validation parameters obtained during validation of developed UHPLC/MS methods allow to use these methods in direct analysis of surface water samples. However, it is unsafe to make a direct injection of surface water into the UHPLC/MS instrument because it may cause a heavy contamination or failure. Therefore, the cheap sample pre-treatment method was developed using bags filled by plant material (leaves of *Reynoutria bohemica*) as a sorbent.

### 3.2 Infrared spectroscopy of used sorbent

Crushed leaves of naturally growing plant *Reynoutria Bohemica* were used as a sorbent in sampling bags. Many studies deal with sorption on the plant material [39-41]. These sorbents are often very cheap and they exhibit good sorption capacities. *Reynoutria Bohemica* (often called simply knotweed) is widespread weed which grows on the banks of rivers and lakes. Cost of this sorbent is therefore very low because mown knotweed is waste. Important advantage of this sorbent utilization is also its fully biodegradable character. But the homogeneity of the sorbent can be considerable problem for sampling purposes because of natural growth of this plant. Therefore, infrared spectroscopy was used for verification of material homogeneity.

Three samples were taken from the bulk of crushed knotweed leaves and their infrared spectra were collected. Spectra are shown at Fig. 6. It can be seen that spectra are almost identical. There are present bands of O-H stretching vibrations (around 3309 cm$^{-1}$), bands of C-H stretching vibrations (2918, 2850 cm$^{-1}$), band of carbonyl stretching vibration (1734 cm$^{-1}$) and several bands of C-O stretching vibrations (1000 – 1100 cm$^{-1}$). Small differences were observed only on intensities of O-H and C-O bands especially around 1032 and 1051 cm$^{-1}$ which indicate some small structural differences among the samples. The differences in C-O vibrations can be probably caused by various esters present in the structure of material. It could be stated according to infrared spectra that used material is homogeneous and it can be used for sorption experiments and also for routine monitoring of the surface water using the developed sampling bag method.

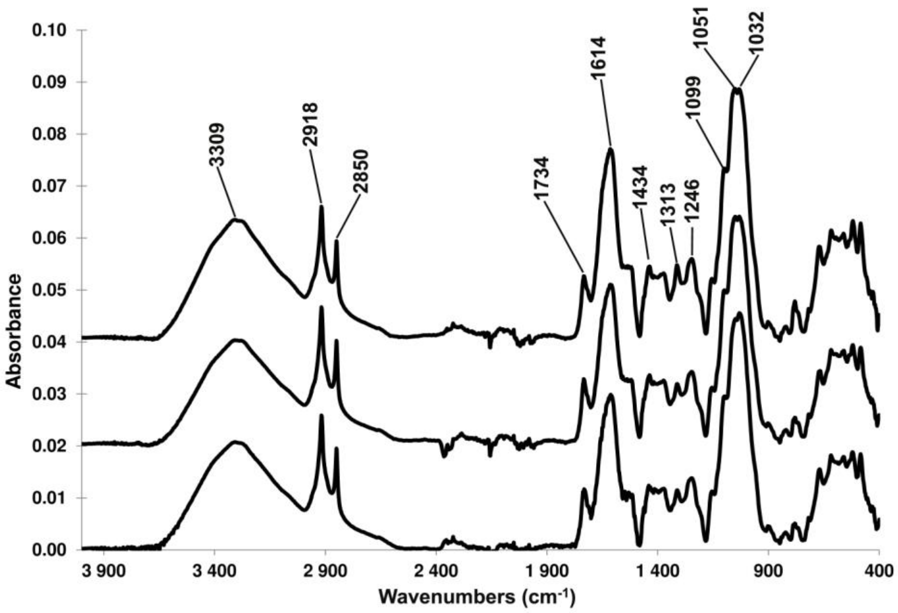

Fig. 6. Infrared spectra of three samples from bulk of used sorbent.

### 3.3 Sorption and desorption properties of sampling bags

The development of sampling bag method is a new direction for routine monitoring of potentially hazardous species in surface waters.

Adsorption and desorption properties of developed sampling bags were determined to describe potential of used sorbent for utilization as sampling agent.

The prepared bags were tested for the maximum possible pharmaceutical sorption capacities (Fig. 7). It can be seen that sorption capacities for individual pharmaceutical are between 37.5 and 77.5 mg/g of sorbent when initial concentration was 400 mg/L (IBU) and 500 mg/L (hormonal contraceptives). These sorption capacities are sufficient for sampling purpose when it is evident from literature [10, 16, 17, 22] that the surface water should contain tens to hundreds ng of studied pharmaceuticals per liter.

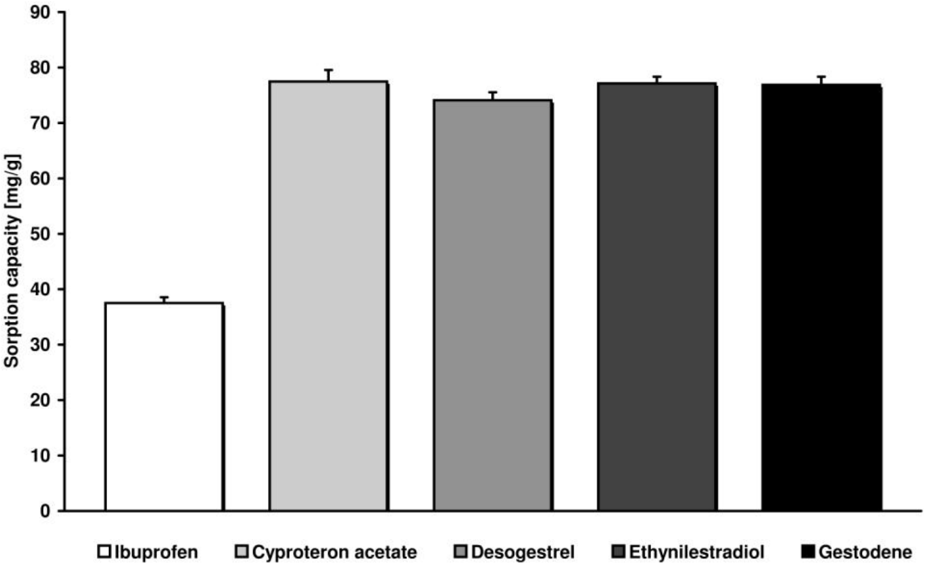

Fig. 7. Sorption capacities of prepared bags [mg/g]. Columns are averages from 5 measurements, lines show standard deviations.

The determination of desorption efficiencies was carried out. Six different types of extraction agents – water (W), acetonitrile (ACN), methanol (MeOH) and mixture of ACN, W and MeOH with v:v:v ratios 50:50:0, 50:0:50 and 0:50:50 (ACN:W:MeOH) were tested. Mixture of ACN:W:MeOH 50:50:0 (v:v:v) exhibits the best desorption efficiency for all 5 pharmaceuticals. This mixture is also suitable for UHPLC/MS system used for final analyses. Desorption efficiencies for selected desorption mixture mentioned above are shown in Table 3. It can be stated that desorption efficiencies are from 60.2 % to 94.3 %.

Table 3 Desorption efficiencies and RSD obtained for 5 measurements

| Pharmaceutical | Desorption efficiency [%] | RSD [%] |
|---|---|---|
| Ibuprofen | 60.24 | 0.03 |
| Cyproteron acetate | 94.31 | 0.04 |
| Desogestrel | 91.70 | 0.03 |
| Ethynidilestradiol | 83.38 | 0.02 |
| Gestodene | 77.17 | 0.02 |

The empty bags were also tested on adsorption and desorption of the selected pharmaceuticals. The obtained results exclude possibility of pharmaceuticals sorption on the bag material.

The pre-treatment of the surface water samples is usually carried out by different types of SPE methods which are characterized by high efficiency but also by high acquisition costs [10, 16, 26, 27]. The new developed sampling method based on sampling bags could be suitable for routine sampling and pre-treatment of the surface water containing Ibuprofen, Ethynilestradiol, Cyproteron acetate, Desogestrel and Gestodene according to sorption capacities of used sorbent for studied pharmaceuticals and also according to desorption efficiencies. However, the developed sampling bag method is less efficient than SPE [10, 16, 26, 27], but our proposed method is much cheaper in comparison with SPE methods and therefore it could be used for routine monitoring of the surface waters contamination wherever it is not necessary to get extremely accurate values of analysis. Moreover, the sampling bag method combines two steps of sampling procedure: a) sample collection and b) pre-treatment into one step. This simplifies the sampling procedure, reduces the risks of mistakes during sampling and minimizes the financial costs in comparison with passive samplers [30].

The sorption capacities of used sorbent (*Reynoutria Bohemica*, knotweed) shows that it could be used also for elimination of pollutants from environment. Various procedures are used for wastewater treatment – for example oxidation [42], biodegradation by microorganisms [43] or reverse osmosis [44]. However, adsorption plays the main role [36, 45-47]. The most used adsorbent is active carbon [36, 45, 47]. The *Reynoutria Bohemica* could be an interesting alternative sorbent for elimination of pharmaceuticals from surface water although the sorption capacity of the studied knotweed does not reach sorption capacities of active carbon [36, 45, 47] but the price of knotweed is much lower than of active carbon.

### 3.4 Sampling method testing in the natural environment

The developed sampling strategy (sampling bag method and instrumental analysis) was tested for monitoring of contamination by studied pharmaceuticals in the Moravian – Silesian surface waters. The sample collection was performed on two rivers and three lakes.

Degree of contamination determined by proposed sampling method is shown in Fig. 8. Units selected for evaluation of possible contamination were ng/g/24h according to used sampling method based on adsorption and due to impossibility of determination of exact volume of water to which the sampling bags were exposed. Data from Fig. 8 show that only tens ng of studied pharmaceuticals were adsorbed at the sorbent in sampling bags per 24 hours. These values are much lower than sorption capacities determined for used sorbent. Therefore, it could be stated that developed sampling bag method is suitable for routine monitoring of contamination in the natural environment.

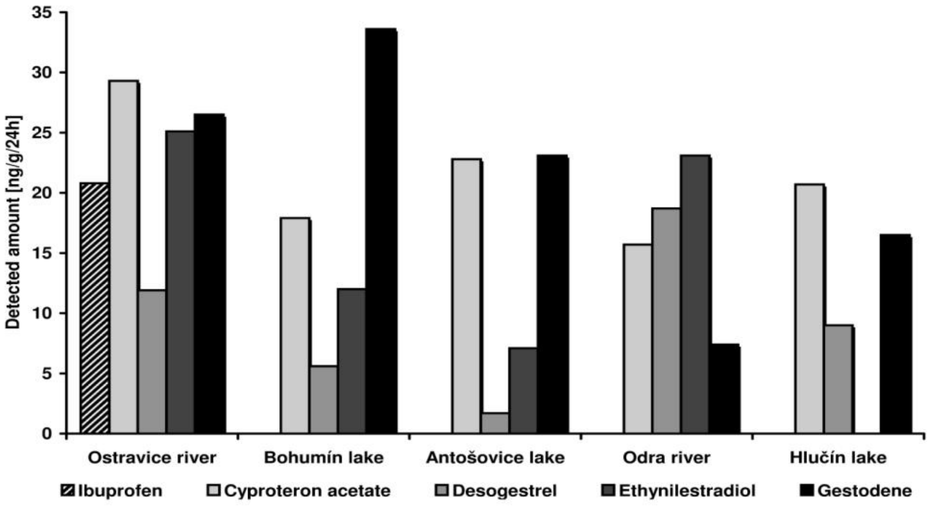

Fig. 8. The detected amounts of pharmaceuticals in the Moravian – Silesian natural waters (bag exposition time – 24 hours, extraction time – 24 hours, analysis by UHPLC/MS methods).

The occurrence of pollutants in the surface water was confirmed by works of Racamonde [10], Fedorova [22] or Patrolleco [16] who detected various pharmaceuticals in the surface waters in the concentration range from tens to hundreds ng/L. Our monitoring confirmed the occurrence of IBU, EES, CA, DES and GES in the surface waters of the Moravian-Silesian Region. The detected amounts represent a relevant risk mainly for water organisms which was confirmed by works of Fedorova [22] or Brozinski [23]. The significant risk is also the pharmaceuticals accumulation in agricultural crops which was demonstrated for example by works of Cortés [20] or Sabourin [21].

Testing of proposed sampling method also led to the suspicion of the drugs presence (for example heroine or pervitine) in Moravian – Silesian surface waters. Unfortunately, this assumption could not be confirmed due to lack of standards at our workplace.

## IV. CONCLUSION

The novel sampling method for routine monitoring of the surface waters contamination and instrumental methods for determination of selected pharmaceuticals were developed. Proposed methods can be applied to the simultaneous determination of Ibuprofen (IBU), Ethynilestradiol (EES), Cyproteron acetate (CA), Desogestrel (DES) and Gestodene (GES) in the surface waters. Two UHPLC/MS methods (one for IBU determination and second for hormonal contraceptives determination) suitable for natural water samples analysis on the concentration level of 1 ng/L were developed and validated. The new sampling bag method was developed to eliminate the risk of direct injection of the surface water into the instrument. Sampling bag method is based on adsorption, therefore sorption capacities and desorption efficiencies of used sorbent (*Reynoutria Bohemica*) to studied pharmaceuticals were determined. Obtained data confirmed that sorbent is suitable for utilization as a sampling agent with sufficient sorption capacity. The developed sampling method was tested for routine monitoring of the Moravian – Silesian surface water contamination. The results of monitoring confirm the presence of the studied pharmaceuticals in selected rivers and lakes.

Proposed novel sampling method do not reach the efficiency of established methods such as SPE but it is suitable for routine monitoring of contamination wherever it is not required extremely accurate values of analysis (for example routine monitoring of surface waters by environmental organizations).

Utilization of proposed sampling method is rather limited at present only to determination of Ibuprofen and four hormonal contraceptives - Ethynilestradiol, Cyproteron acetate, Desogestrel and Gestodene. Therefore, the goal for the future research is to extend the group of determinable analytes.

## V. ACKNOWLEDGEMENTS

This study was supported by the Project LO1208 (TEWEP) of the National Feasibility Programme I of the Czech Republic, by the student grant of University of Ostrava with identification number sgs01/PřF/2015 and by the project of the Moravian-Silesian Region reg. no. 02679/2014/RRC. The authors thank to dr. Martin Mucha for help with IR spectra measurement and interpretation.

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