MONITORING OF SOME PHARMACEUTICAL COMPOUNDS IN WASTE WATER TREATMENT PLANT INFLUENT AND EFFLUENT SAMPLES BY LIQUID CHROMATOGRAPHY

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Abstract

Pharmaceutical compounds are continually introduced into the environment as a result of industrial and domestic use. Influents and effluents from three municipal waste water treatment plants (WWTPs) with varying waste water treatment technologies and design were analyzed for six anti-inflammatory drugs, an antiepileptic drug and a nervous stimulant during nine months period. The temporal evolution and removal rates in the WWTPs of the pharmaceutical compounds have been studied. Analytical determination was carried out by high performance liquid chromatography (HPLC) with multiwaved detector after sample clean up and concentration by solid phase extraction (SPE). All compounds were detected not only in influents but also in waste water effluents. These findings indicate that several conventional waste water management practices are not effective in the complete removal of pharmaceutical compounds, and their discharges have a large potential to affect the aquatic environment.

Keywords: *monitoring, pharmaceuticals, HPLC, WWTP, waste water*

Introduction

In recent years, the occurence and fate of pharmaceutical compunds in the aquatic environment has raised great concern. The amount of pharmaceutical compounds being introduced into the environment is likely low. However, due to their continuous introduction into the environment and synergic effects through combined parallel action, even compounds of a low persistence might cause adverse effects on aquatic and terrestrial organisms /1/. Numerous papers reported the level of pharmaceuticals in wastewater, aqueous and solid environmental matrices. Wastewater treatment plants have been identified as a major source of pharmaceutical compounds entering the environment. Elimination of these compounds in a conventional wastewater treatment processes is often low and consequently, compounds not removed are released to receiving water bodies in WWTP effluent streams /2-4/. Therefore, effluents from WWTPs can be considered one of the most important sources of pharmaceuticals in the environment. Antibiotics, anti-inflammatory, antiepileptic drugs are some of the most representative pharmaceutical compounds found in WWTPs influents and effluents. Extensive researchs regarding the concentrations of pharmaceuticals in the environment has focused on their occurence in surface water samples affected by WWTPs effluents /5-7/. Due to

their high water solubility and often poor degradability pharmaceuticals are able to penetrate through all natural filtration steps and enter groundwater as well as drinking water /8-10/. Environmental contamination with pharmaceuticals as a result of WWTPs effluent discharges depends on several factors such as the nature of the target pollutants, the type of wastewater treatment processes emplyed, age of the activated sludge and climatic conditions. The main objective of the research presented in this paper was to verify the occurence and fate of some anti-inflamatory drugs (acetaminophen, carbamazepine, ketoprofen, naproxene, diclofenac, indomethacine, ibuprofen), caffeine and an neutral antiepileptic drug (carbamazepine) in WWTPs influent and effluent samples. Analysis of target compounds was performed by a previously reported validated method based on sample preparation by solid-phase extraction and HPLC-MWD determination /11,12/. The removal rates of these compounds in the WWTPs studied are also reported.

Experimental

Chemicals and reagents

Carbamazepine, diclofenac, ibuprofen, ketoprofen, naproxen, indomethacine and acetaminophen (98 – 99.9 % purity) were all purchased from Sigma-Aldrich (Steinheim, Germany). Caffeine was obtained from Merck (Darmstadt, Germany). HPLC-grade acetonitrile, methanol, water, and ethyl acetate as well as potassium dihydrogen phosphate (99,995 % purity) were supplied by Merck (Darmstadt, Germany). Oasis HLB cartridges (60 mg/3 mL) used for solid phase extraction were purchased from Waters (Milford, Massachusetts, USA). Glass microfibre filters were purchased from Whatman (United Kingdom). Stock standard solutions of about 500 µg mL^{-1} were prepared in methanol. The individual dilutions and mixtures of the analytes were prepared in the same solvent. All stocks and diluted standard solutions were protected against light in amber vials and were stored at 4 °C.

Sample collection

Influent and effluent samples were collected monthly from three wastewater treatment plants in the Pitesti, Brasov and Targu Mures area. Treatments in all of the WWTPs studied included primary (settling and flotation) and secondary (activated sludges) conventional treatments. Amber glass bottles were used to collect momentary samples from each site. Each bottle was filled to the top to reduce headspace and transported to the laboratory. Samples were stored at 4 °C until analysed. All samples were analysed within 3 days. Fifty four influent and effluent samples were collected from January till September 2011.

Solid-phase extraction procedure

Prior to extraction, 1000 mL of influent or effluent wastewater were filtered through 0.45 µm Whatman glass fibre filters to remove any solid particulates and adjusted to pH 2 with hydrochloric acid (2M) in order to prevent the analytes from taking their ionic form. Conditioning of the SPE cartridges was performed with 3 mL ethyl acetate, 3 mL of methanol and 3 mL of acidified water (pH 2) at a flow rate of 5 mL/min. After loading the sample and

subsequent washing with 5 mL of HPLC water at 5 mL/min, the cartridges were dried under vacuum for 30 min. The elution of analytes was performed with 3 mL ethyl acetate at a flow-rate of about 1 mL/min. The extract was evaporated to dryness in a nitrogen stream and finally reconstituted in 1 mL of methanol and injected into the HPLC system.

Liquid chromatographic separation

Analytical determination was performed on an Agilent 1100 (Agilent Technologies, USA) system equipped with a degasser, quaternary pump, autosampler, column thermostat and multiple wavelength detector (MWD). The separations were performed on a LiChrosphere® 100 RP-18 analytical column (125 mm length, 4 mm i.d; 5 µm particle size) acquired from Merck (Darmstadt, Germany) protected by a LiChrosphere® 100 RP-18 (4 mm x 4 mm i.d., 5 µm) guard column. System control and data acquisition were achieved by means of a computer equipped with an Agilent ChemStation program. Analytes were separated by gradient elution using 50 mM potassium dihydrogen phosphate in water (pH 4.6) (solvent A) and acetonitrile as mobile phase (solvent B) at a flow-rate of 1 mL/min. The HPLC separations were carried out at 25 °C and a 20 µL injection volume was employed for this assay. The linear gradient program is listed in Table 1. The detection was performed at 220 nm for indomethacine and ibuprofen, 230 nm for naproxen, 248 nm for acetaminophen, 254 for ketoprofen and 285 nm for diclofenac, which were determined to be the optimum wavelengths in preliminary studies performed on a scanning spectrometer. Peak area were used for quantitative analyses. Compounds were identified in the chromatograms comparing the retention time of the peaks with that of the corresponding compounds in the standard solution.

Table 1. Eluent gradient

Time (min)	Solvent A (%)	Solvent B (%)
0	85	15
4	85	15
9	75	25
19	55	55
35	60	40

Solvent A: 50 mM potassium dihydrogen phosphate; solvent B: acetonitrile

Removal rates in the WWTPs

Removal rates of the monitored pharmaceutical compounds in the WWTPs were calculated with the following equation: $(C_i - C_e) \times 100/C_i$ where C_i is the concentration of the analyte measured in the influent sample and C_e is the concentration of the analyte measured in the corresponding effluent sample.

Results and discussion

Occurrence of pharmaceuticals in WWTPs influents and effluents

All monitored pharmaceutical compounds were found in the influents and effluents from the three WWTPs studied in concentrations higher than the limits of detection of the method. Their concentration range, mean and Relative Standard Deviation (RSD) are presented in the Table 2.

Table 2. Concentrations of the analyzed pharmaceutical compounds in the three WWTPs

Pharmaceutical compound/ WWTPs	Influent			Effluent		
	Concentration range (µg/L)	Mean (µg/L)	RSD (%)	Concentration range (µg/L)	Mean (µg/L)	RSD (%)
Acetaminophen						
Pitesti	6.00-29.59	14.37	71.89	0.51-29.34	6.68	137.23
Brasov	0.41-24.56	13.12	61.90	0.08-17.55	7.22	81.36
Tg-Mures	0.29-32.18	9.78	94.02	0.19-2.80	1.03	86.74
Caffeine /						
Pitesti	4.03-32.22	20.39	40.64	0.21-20.94	5.64	126.87
Brasov	2.05-37.06	22.34	44.83	1.28-25.49	17.03	48.43
Tg-Mures	3.57-37.73	20.69	54.74	<0.06-14.21	1.81	74.53
Carbamazepine/						
Pitesti	0.07-5.24	0.89	182.73	<0.04-0.51	0.14	121.85
Brasov	<0.04-9.46	1.46	220.75	<0.04-6.39	0.93	236.46
Tg-Mures	0.05-6.96	1.27	169.15	<0.04-0.22	0.15	31.74
Ketoprofen/						
Pitesti	0.66-18.14	7.03	74.01	0.25-4.87	2.97	94.04
Brasov	1.26-21.66	9.20	67.77	0.33-4.24	2.13	54.78
Tg-Mures	0.15-10.51	3.17	102.6	0.19-1.47	0.68	59.87
Naproxene/						
Pitesti	<0.04-0.68	0.28	74.78	<0.04-0.37	0.10	125.46
Brasov	<0.04-0.67	0.23	87.37	<0.04-0.24	0.13	51.61
Tg-Mures	0.13-0.74	0.40	52.89	<0.04-0.50	0.16	119.85
Diclofenac/						
Pitesti	2.30-22.04	8.20	79.65	0.65-16.68	4.62	108.82
Brasov	0.43-13.34	6.96	65.69	0.30-5.40	2.72	70.42
Tg-Mures	2.14-10.59	5.96	52.97	0.55-3.36	2.12	53.93
Indomethacine/						
Pitesti	<0.06-3.37	1.52	82.79	<0.06-1.75	0.78	87.56
Brasov	<0.06-3.63	1.52	75.61	<0.06-0.84	0.23	145.89
Tg-Mures	0.11-3.80	1.64	78.28	<0.06-0.58	0.27	66.29
Ibuprofen/						
Pitesti	<0.16-99.33	34.55	106.82	<0.16-25.84	11.41	73.01
Brasov	<0.16-31.20	18.97	63.87	<0.16-20.00	10.60	79.24
Tg-Mures	0.22-35.13	10.93	101.96	<0.16-1.55	0.81	54.39

As can be seen in the Table 2, all of the pharmaceutical compounds monitored were detected in the wastewater samples analyzed. Acetaminophen was detected in all wastewater samples and its concentrations varied from 0.08 to 32.18 µg/L with mean concentrations of 12.42 and 4.98 µg/L in influents and effluents, respectively. Caffeine was detected in 98% of the samples, the highest concentration value being 37.73 µg/L. Carbamazepine was quantified in most cases at concentration levels lower than 1.00 µg/L excepting April month with higher concentrations. Ketoprofen was also detected in all of the wastewater samples analyzed in a concentration range from 0.15 to 21.66 µg/L with mean concentrations of 6.46 and 1.93 µg/L in influents and effluents, respectively.

Naproxene and indomethacine were detected in 79% and 70% of the analyzed samples, in concentrations lower than 1.00 µg/L. The concentrations of diclofenac detected in all the samples ranged from 0.30 to 22.04 µg/L. The highest values of concentrations of investigated pharmaceuticals were found for ibuprofen in Pitesti WWTP influent of 99.33 µg/L in April and 84.26 µg/L in July. Ibuprofen was the pharmaceutical compound present at the highest concentration level in all wastewater samples, suggesting its widespread and frequent use. These results are in agreement with data reported by other authors in wastewater samples from Sweden /13/ and Spain /14,15/.

Temporal evolution of the pharmaceuticals during the sampling period

The temporal evolution of the concentration of the pharmaceutical active compounds in influent and effluent wastewater during the monitoring period can be seen in Fig. 1-3. Each point shows the momentary concentration of the collected sample. An increased concentration in influent and effluent wastewater of ibuprofen, acetaminophen, caffeine, diclofenac and ketoprofen was observed in the coldest period of the year which correspond to the period January-March (months 1-3) and that can be associated to the increase of consumption. Generally, the lowest concentrations of the pharmaceuticals were recorded in June and July in all the three WWTPs studied. No seasonal influence was observed in the concentration of carbamazepine, naproxen and indomethacine in WWTPs influent and effluent wastewater samples studied.

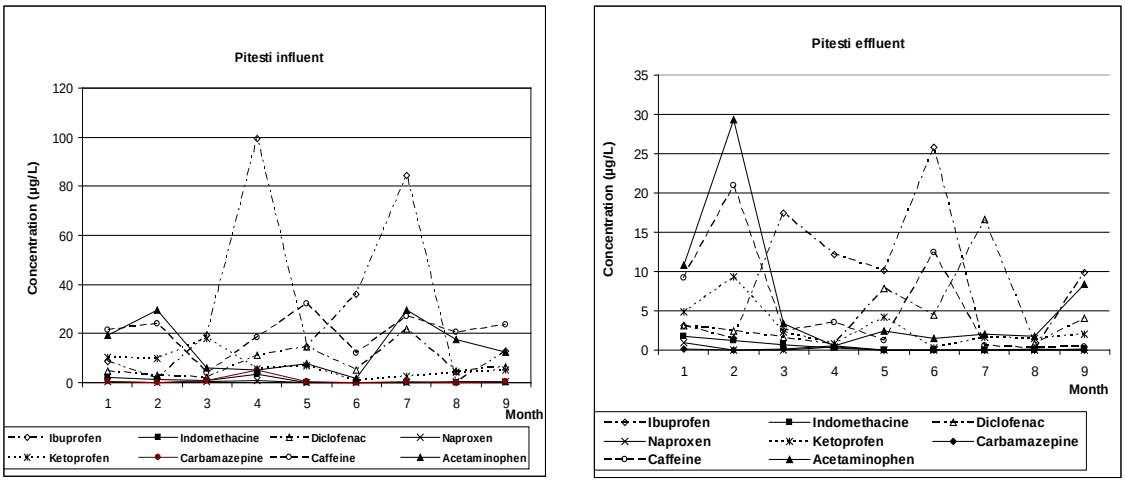

Fig. 1. Temporal evolution of the pharmaceuticals concentrations in the Pitesti WWTPs during 9-months monitoring period.

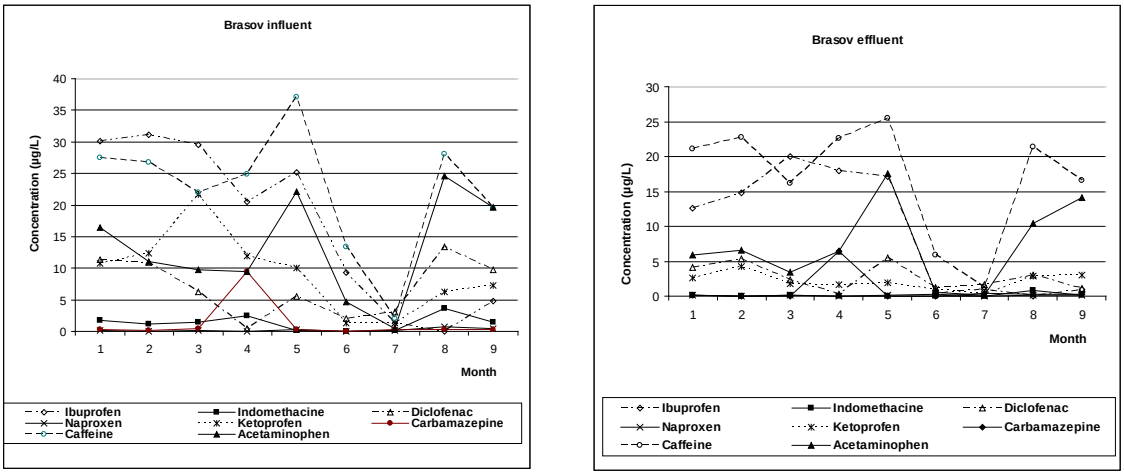

Fig. 2. Temporal evolution of the pharmaceuticals concentrations in the Brasov WWTPs during 9-months monitoring period.

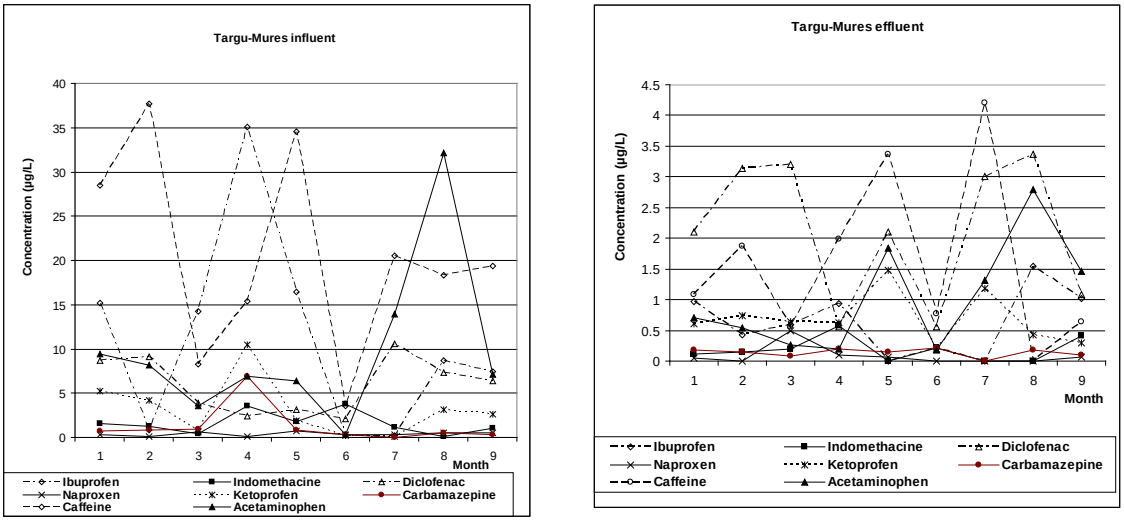

Fig. 3. Temporal evolution of the pharmaceuticals concentrations in the Targu Mures WWTPs during 9-months monitoring period.

Removal of the pharmaceutical compounds in the WWTPs

Removal rates of each pharmaceutical compounds in each WWTP studied during the nine months monitoring period are presented in Fig. 4. The investigated compounds showed different removal rates from one WWTP to the next. Removal rates of acetaminophen ranged between 37 % and 90%. Removal rates ranging from 75% to 86% were observed for naproxen, and for indomethacine from 56% to 94%. It was reported that analgesics such as naproxen and ibuprofen are removed from wastewater mainly due to their bio-degradability /16/. Partial removal of carbamazepine, a compound with poor biodegradability at low concentration, was observed in Targu Mures (20%) and

Brasov (9%) WWTPs, while negligible removal of only 1% was observed in Pitesti WWTP. These results are in agreemet with the low removal rates previously presented in other reports /17,18/. The highest removal rate of 98% was observed for caffeine in the Pitesti WWTP in August. Removal rates of ketoprofen and diclofenac varied significantly between WWTPs in the ranges 24-76% and 48-84%, respectively. Similar removal efficiencies of ketoprofen and diclofenac were reported at different WWTPs /19 /.

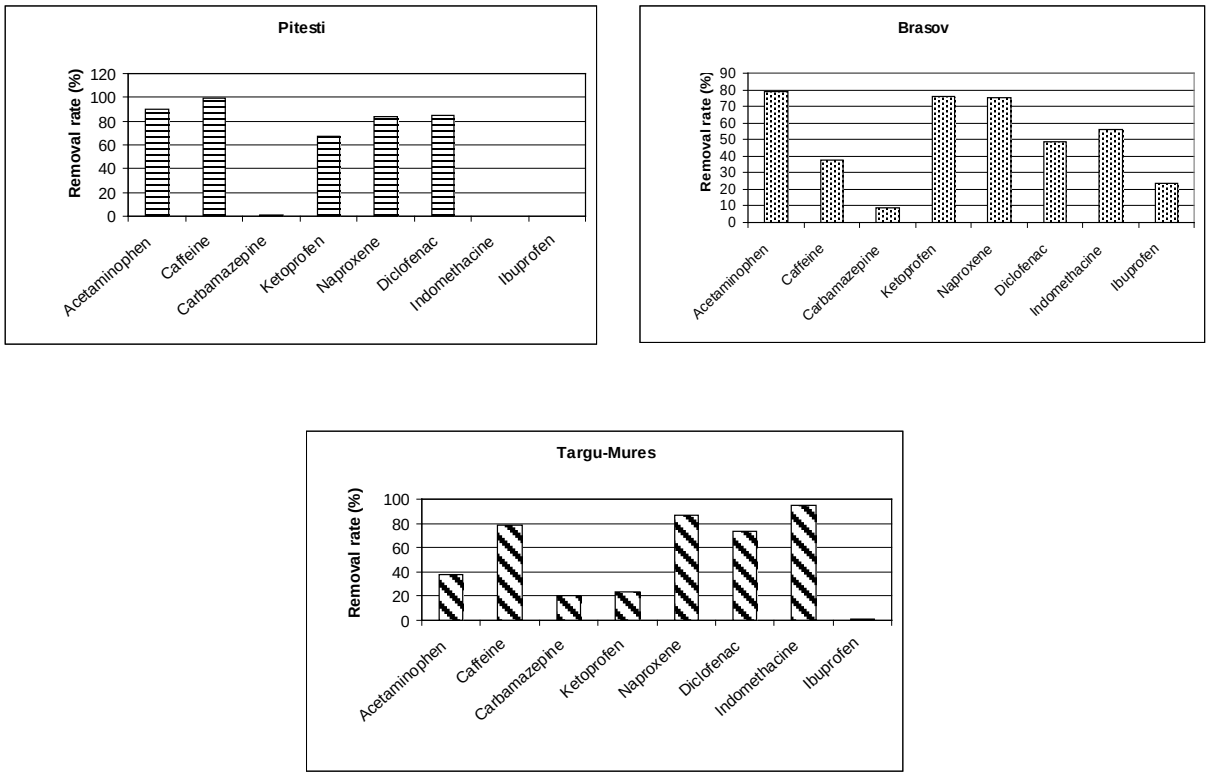

Fig. 5. Removal efficiencies (%) of the investigated pharmaceuticals in the three WWTPs

Conclusions

A previously developped method has been used for the simultaneous analysis of some anti-inflammatory drugs, caffeine and carbamazepine in influent and effluent wastewater samples collected from three WWTPs during a nine months period from January till September 2011. All of the pharmaceutical compounds were detected in influent and effluent wastewater samples. The highest concentration levels were recorded for Ibuprofen of 99.33 µg/L. A seasonal evolution of the concentration of some of the investigated compounds was observed. Although wastewater treatments were similar in all of the WWTPs evaluated, different removal efficiencies were observed. Lower removal rates were observed for carbamazepine ranging from 1.08% to 20.22%. Removal rates of the other pharmaceuticals were in range from 23% to 98%.

References

1. Fent, K., Weston, A.A., Caminada, D., *Aquat Toxicol*, 2006, 76, 122-159.
2. Lacey, C., McMahon, G., Bones, J., Barron, L., Morrissey, A., Tobin, J.M., *Talanta*, 2008, 75, 1089-1097.
3. Kumerer, K., *Chemosphere*, 2001, 45, 957-969
4. Batt, A.L, Aga, D. S., *Anal. Chem*., 2005, 77, 2940-2947
5. Westerhoff, P., Yoon, Y.m., Snyder, S., Wert, E., *Environ. Sci. Technol*., 2005, 39, 6649-6663.
6. Vieno, N.M., Härkki, H., Tuhkanen, T., Kronberg, L., 2007*, Environ. Sci. Technol.,* 2007, 41, 5077-5084.
7. Gómez, M.J., Martínez Bueno, M.J., Lacorte, S., Fernández-Alba, A.R., Agüera, A., *Chemosphere,* 2007, 66, 993-1002.
8. Sacher, F., Lange, F.Th., Brauch, H.-J., Blankenhorn, I., *J. Chromatogr. A,* 2001, 938, 199-210.
9. Heberer, Th., Stan, H.J., *Int. J. Environ. Anal. Chem*., 1997, 67, 113-124.
10. Heberer, Th., Reddersen, K., Mechlinski, A. *Water Sci. Technol*., 2002, 46, 81-88
11. Petre, J., Vasile I.I., Staniloae, D., Nastac, E., Cruceru, L., International Symposium "The Environment and Industry" 28-30 October 2009, Bucharest, Romania.
12. Petre, J.,Vasile I.I., Cruceru, L., Nastac, E., The XXXI-st Romanian Chemistry Conference, 6-8 October Rm. Valcea 2010.
13. Bendz, D., Paxéus, N.A., Ginn, T.R., Loge, F.J., *J. Hazard Mater*, 2005, 122, 195-204.
14. Carballa, M., Omil, F., Lema, J., Llompart, M., García-Jares, C., Rodríguez, I, Gómez, M.J., Ternes, T.A., *Water Res,* 2004, 38, 2918-2926.
15. Santos, J.L., Aparicio, I., Callejón, M., Alonso, E., *Journal of Hazardous Materials*, 2009, 164, 1509-1516.
16. Kasprzyk-Hordern, B., Dinsdale, R.M., Guwy, A.J., *Water Res*., 2009, 43, 363-380.
17. Joss, A., Keller, E., Alder, A.C., Gobel, A., McArdell, C.S., Ternes, T.A., *Water Res*., 2005, 39, 39-52.
18. Santos, J.L., Aparicio, I., Alonso, E., *Environ. Int*., 2007, 33, 596-601.
19. Lindquivist, N., Tuhkanen, T., Kronberg, I., *Water Res*., 2005, 39, 2219-2228.