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Occurrence and analysis of endocrine-disrupting compounds in a water supply system

A. R. M Carvalho · V. V. Cardoso · A. Rodrigues · E. Ferreira · M. J. Benoliel · E. A. Duarte

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Abstract This paper presents the study of the occurrence of 10 endocrine-disrupting compounds (EDCs) in 60 water samples using a method for simultaneous quantification and confirmation of the presence of these emerging compounds, using ultra-performance liquid chromatography with electrospray ionization and *tandem* mass spectrometry (UPLC-ESI-MS/MS). All samples were previously extracted by solid-phase extraction (SPE). Several natural and synthetic hormones (17-β-estradiol, ethinylestradiol, estriol, estrone, progesterone, mestranol, and diethylstilbestrol) and some industrial products (4-*n*-nonylphenol, 4-*tert*-octylphenol, and bisphenol A) were chosen for this survey. The analytical limits were calculated for each compound and were used in the identification and quantification of these target compounds in EPAL's water supply system. In this study, several samples were taken from the main intakes of water (surface and groundwater) used for production of water for human consumption and from different sampling points of the drinking water distribution system (piping, nets, and reservoirs). Some target compounds, such as estriol, 4-*tert*-octylphenol, mestranol, and nonylphenol, were found in trace amounts in several water samples. However, the studied endocrine-disrupting appeared in very low concentrations when compared with the assessed analytical limits.

Keywords Water supply system · Endocrine-disrupting compounds · Ultra-performance liquid chromatography · Solid-phase extraction · Environmental health

Introduction

In recent years, a large number of organic pollutants have been found worldwide in surface waters, groundwater, and soils, among other matrices. Yet, there are still many unanswered questions about their transport, fate, and cumulative impacts throughout environmental compartments (Stanford and Weinberg 2007; López-Roldán et al. 2010). The presence of these substances raises alarm especially when water is used for drinking water production, but environmental health should also be considered.

Endocrine-disrupting chemicals (EDCs) are a heterogeneous group of substances characterized by their potential to interfere with endocrine system functions in wildlife and humans (San Filippo et al. 2010; Fuhrman et al. 2015). The presence of these contaminants in aquatic environment and their potential effects on living organisms have become a growing concern in recent years, which requires identification, analysis, and characterization of risks in different environmental matrices (Dolar et al. 2012; Fuhrman et al. 2015; Fredj et al. 2015). The continuous use of these substances raises

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A. R. M. Carvalho (✉) · E. A. Duarte
Departamento de Ciências e Engenharia dos Biossistemas (DCEB), Instituto Superior de Agronomia, Tapada da Ajuda, 1349-017 Lisbon, Portugal
e-mail: anaritam_carvalho@hotmail.com

V. V. Cardoso · A. Rodrigues · E. Ferreira · M. J. Benoliel
Direcção de Controlo de Qualidade da Água, Laboratório Central, Empresa Portuguesa de Águas Livres, S.A. – EPAL, Avenida de Berlim, 15, 1800-031 Lisbon, Portugal

the concern about which are the behavior, environmental fate, and the potential adverse effects on nontarget organisms of these compounds once incorporated into the environment (Esteban et al. 2014).

According to 2013/39 Directive, of the European Parliament and of the Council of 12 August, which states on environmental quality standards in the field of water policy, only nonylphenol and octylphenol have annual average-environmental quality standards (AA-EQS). AA-EQS for these priority substances are 0.30 and 0.10 μg L^{-1}, respectively. The precautionary principle of Drinking Water Directive (Council Directive 98/83/EC of 3 November of 1998, concerning the quality of water intended for human consumption) establishes that for compounds without guideline values a concentration limit value of 0.10 μg L^{-1} will be assumed.

Accurate research using sensitive and sophisticated chemical analytical methods to detect and quantify emerging compounds at low concentrations at which they occur in the environment were assessed in the last decade (Kosjek et al. 2005; Sebők et al. 2009; Kasprzyk-Hordern et al. 2008; Boles and Wells 2010).

This study allowed the quality characterization of different types of water samples, taking into account the levels of the studied target compounds found in the samples and is focused in the assessment of the occurrence of 10 endocrine-disrupting compounds (EDCs) in the water supply system from Empresa Portuguesa das Águas Livres, S.A (EPAL).

The main objective of this work was to use the SPE-UPLC-ESI-MS/MS method to provide a more extensive knowledge concerning the trace organic contamination in water, encompassing some strategic locations in a drinking water system. The results obtained are valuable information to confirm the usefulness of the method and to ensure compliance between the founded concentration values of each EDC and the limits established by law.

Material and methods

Empresa Portuguesa de Águas Livres

Empresa Portuguesa das Águas Livres (EPAL, S.A.) is the largest drinking water supply company in Portugal and is responsible for the production and supply of drinking water to the city of Lisbon, and to 35 municipalities around Lisbon. The production system includes two conventional water treatment plants (WTP1—Asseiceira and WTP2—Vale da Pedra) which catch and treat surface water from Castelo de Bode dam and Tagus River, respectively. WTP1 has the following schedule procedure: pre-oxidation (if necessary), coagulation/flocculation with aluminum sulfate, flotation, filtration, pH adjustment, and final disinfection. WTP2 has the following schedule procedure: pre-oxidation, pH adjustment and remineralization, coagulation/flocculation with aluminum sulfate and polyacrylamide, decantation, sand filtration, pH adjustment, and final disinfection. Chlorine is used in the pre-oxidation and in the final disinfection steps of water in the two WTP. Along the supply system, the water is rechlorinated at 27 injection points, in order to guarantee residual chlorine at Lisbon consumers' tap. It also has a spring water source in Olhos de Água and 19 groundwater sources. An intensive drinking water quality control program is performed by EPAL Central Laboratory throughout the water supply system, from water sources to consumers' tap water in the city of Lisbon (Penetra et al. 2010).

Monitoring sampling campaign

The monitoring sampling campaign was carried out between February and April 2012 evolved 60 samples from EPAL's water supply system, encompassing raw water (surface and groundwater catchments), pumping stations, reservoirs, and one sampling points of the water distribution system. This campaign evolved the collection of 13 samples from the catchment areas (12 samples from groundwater catchments and 1 from a superficial catchment), 44 samples from reservoirs, 2 samples from pumping stations, and 1 chlorinated water sample. A schematic diagram of the water supply system showing the sampling sites is illustrated in Fig. 1.

Target compounds

Ten EDCs were analyzed in water supply system samples, including natural hormones (estriol, 17-β-estradiol, and estrone), synthetic hormones (ethinylestradiol, progesterone, mestranol, and diethylstilbestrol), and some industrial products (4-*n*-nonylphenol, 4-*tert*-octylphenol, and bisphenol A), using one of the best options for targeted analysis, ultra-performance liquid chromatography coupled to mass spectrometry using a triple quadrupole spectrometer (UPLC-ESI-MS/MS).

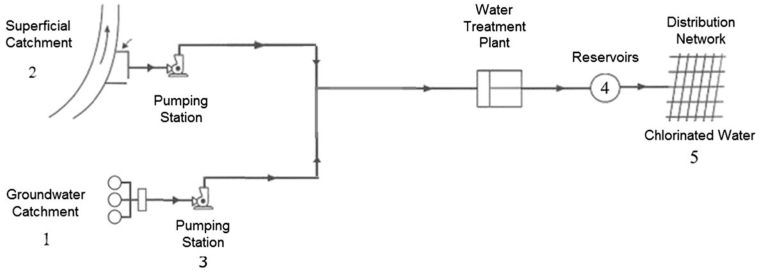

Fig. 1 Sampling points along the water supply system: *1* groundwater catchment; *2* superficial catchment; *3* pumping station; *4* reservoirs; *5* chlorinated water

Before instrumental analysis, the samples in study were pretreated by solid-phase extraction (SPE).

Sample preparation and analytical determination

EDCs were analyzed on the samples previously prepared by SPE followed by UPLC-ESI-MS/MS, according with Carvalho (2012). Figure 2 synthetizes samples preparation and analyses steps applied in this study.

Water samples were collected in 1000-mL glass amber bottles and transported to the laboratory under cooled conditions (4 °C) and were analyzed within 48 h after sampling.

Individual standards of each EDC were obtained from Dr. Ehrenstorfer GmbH, Germany. The purity of these standards was analytical grade (<90 %).A mixture of all EDC standards was prepared by appropriate dilution of the individual stock solutions and was used to the preparation of several working standard solutions for external calibration.

The validated SPE-UPLC-ESI-MS/MS method (ME 72 2010), an internal method of EPAL, was applied to the analysis of the 10 endocrine-disrupting compounds in 60 samples from a water supply system.

Samples were previously prepared by solid-phase extraction (SPE), using a Thermo Scientific™ Dionex™ AutoTrace™, 280 Solid-Phase Extraction (SPE) equipment. The cartridges used in SPE procedure were SPE Waters Oasis HLB, 6 mL, 200 mg.

The chromatographic analysis was performed on a liquid chromatograph Waters ACQUITY UPLC, equipped with a quaternary pump and an autosampler. This equipment was connected to a triple quadrupole mass spectrometer Waters ACQUITY TQD (API), equipped with electrospray ionization (ESI) source, from Waters (Milford, MA, USA).

The software used for instrumental control and data acquisition was MassLynx version 4.0, 2002 from Micromass.

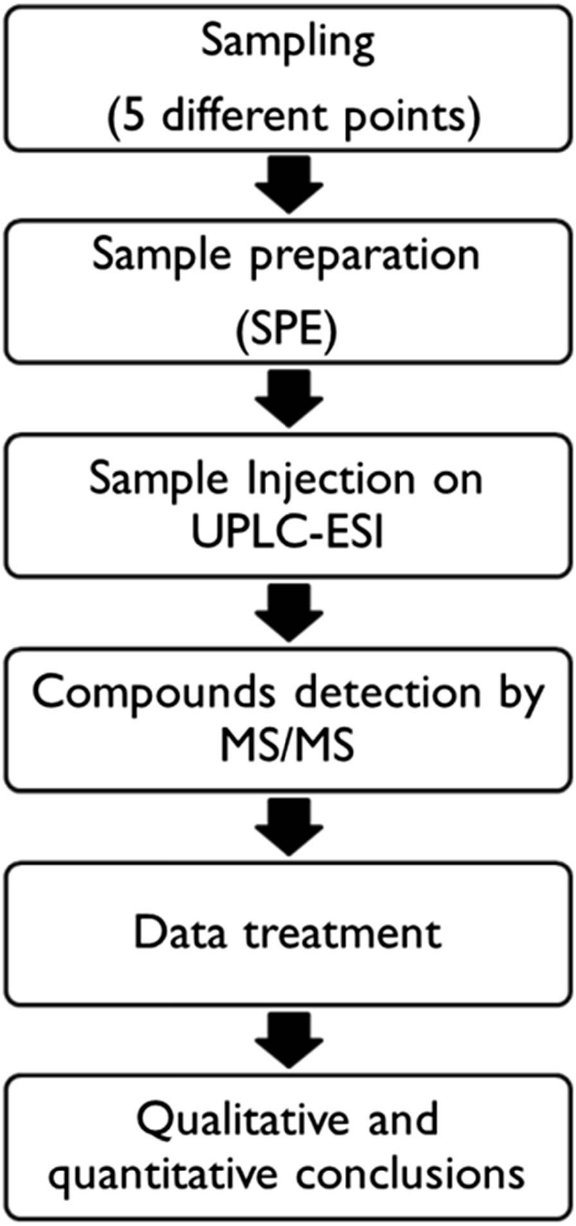

Fig. 2 Samples preparation and analysis steps

The chromatographic separation was performed with an ACQUITY BEH C18 (2.1×50 mm, 1.7 μm) column from Waters (Milford, MA, USA), using 0.05 % (*v*/*v*) of ammonia in water and methanol as mobile phase.

The injection volume was 20 μl, the flow rate was kept constant at 0.5 mL min^{-1} under gradient conditions, and the temperature of the column compartment was set to 40 °C.

The optimized MS/MS parameters (cone voltage and collision energy) and MRM transitions for each target compound are listed in Table 1.

The detailed method procedure could be access in Carvalho (2012).

Quality assurance and quality control

Linearity (L), limits of detection (LOD) and quantification (LOQ), recovery, and repeatability (R) were investigated during this study. Analysis of each water sample was performed in triplicate and was accompanied by the analysis of one blank laboratory reagent. Limit of detection (LOD) and limit of quantification (LOQ) are two fundamental parameters to determine if an analyte is present in the sample. The LOD is the minimum detectable amount of analyte in the sample, while the LOQ is the minimum amount that could be quantified, and they were evaluated using the standard deviation of various readings of the solution with the lowest concentration of the linearity range (S0).

Analytical limits were calculated for each compound under study. LOD corresponds to three times the value of S0 and LOQ corresponds to 10 times the value of SO and both analytical limits are expressed in nanogram per liter. The concentration values of each EDC were obtained through the linearization of the function that correlate the concentration value with each peak area.

Physico-chemical properties and analytical limits of each target compound

This paper focuses on 10 EDCs with different properties. Table 2 shows their physico-chemical properties and the detection and quantification limits of each EDC.

Results and discussion

The main results of each target compound's concentrations obtained are presented in Fig. 3. This figure shows the average concentration (ng L^{-1}) of each EDC in each sampling point.

Observing Fig. 3a, it is clearly visible that trace amounts of all compounds were found in a universe of 13 groundwater samples, except estradiol, ethinylestradiol, and progesterone. However, only the industrial product nonylphenol were detectable in one sample above the method LOD (7.17 ng L^{-1}), having an estimated concentration value of 8.12 ng L^{-1}.

Figure 3b represents concentration values of each studied EDCs in one sample collected in a surface water catchment (Tagus River). None of the 10 compounds was detected or quantified in this sample. However, it is important to consider that only one sample is not representative of this matrix and further data is needed to

Table 1 Monitoring conditions—MRM used in the detection of EDC

EDC	Ionization mode	Precursor ion	Cone voltage (V)	Product ion quantification (MRM1)	Collision energy (eV)	Product ion qualification (MRM2)	Collision energy (eV)
Estriol ($C_{18}H_{24}O_3$)	ES −	287.4	60	145.4	40	171.3	40
Bisphenol A ($C_{15}H_{16}O_2$)	ES −	227.4	40	212.3	20	133.2	20
Diethylstilbestrol ($C_{18}H_{20}O_2$)	ES −	267.3	40	251.4	25	237.4	25
Estrone ($C_{18}H_{22}O_2$)	ES −	269.3	60	145.2	40	143.0	55
17-β-estradiol ($C_{18}H_{24}O_2$)	ES −	271.4	65	145.3	40	143.0	65
Ethinylestradiol ($C_{20}H_{24}O_3$)	ES −	295.4	50	145.0	39	159.0	34
Progesterone ($C_{21}H_{30}O_2$)	ES +	315.4	50	96.9	20	108.9	25
Octylphenol ($C_{14}H_{22}O$)	ES −	205.2	20	133.8	15	132.8	20
Mestranol ($C_{21}H_{26}O_2$)	ES +	311.4	30	121.2	20	159.2	15
Nonylphenol ($C_{15}H_{24}O$)	ES −	219.4	40	119.0	22	133.3	30

Table 2 Physico-chemical properties and analytical limits of each target compound

	Molecular weight (g mol^{-1})	CAS number	LOD (ng L^{-1})	LOQ (ng L^{-1})
Estriol ($C_{18}H_{24}O_3$)	288.39	50-27-1	6.82	20.5
Bisphenol A ($C_{15}H_{16}O_2$)	228.29	80-05-7	6.29	18.9
Diethylstilbestrol ($C_{18}H_{20}$O2)	268.36	8053-00-7	6.54	19.6
Estrone ($C_{18}H_{22}O_2$)	270.37	53-16-7	7.24	21.7
17-β-estradiol ($C_{18}H_{24}O_2$)	272.39	82115-62-6	6.86	20.6
Ethinylestradiol ($C_{20}H_{24}O_3$)	296.41	77538-56-8	6.83	20.5
Progesterone ($C_{21}H_{30}O_2$)	314.46	9002-68-0	6.87	20.6
Octylphenol ($C_{14}H_{22}O$)	206.33	67632-66-0	7.03	21.1
Mestranol ($C_{21}H_{26}O_2$)	310.43	72-33-3	6.73	20.2
Nonylphenol ($C_{15}H_{24}O$)	220.34	68152-92-1	7.17	21.5

characterize the prevalence of EDCs in this surface water.

Figure 3c, related to the analysis of two samples collected in pumping stations, show the detection of estriol and nonylphenol, a natural hormone and an industrial product, respectively, with values between 9.85 ±0.51 and 19.2±3.99 ng L^{-1}. Comparing these values with the estimated method LOD and LOQ of these two compounds, it was possible to confirm the presence of estriol in two of the samples, but only nonylphenol could be quantified in one of the samples, once this value was above the LOQ. The results obtained for the treated waters analyzed confirm the suitability of the water treatment process applied in the waterworks investigated: all the monitored compounds are either completely eliminated or, as in the case of estriol and nonylphenol, removed to a level below the maximum legislated value of 0.1 μg L^{-1} in the final drinking water.

Concerning the reservoirs sampling point, for a total of 44 samples, Fig. 3d shows that six EDCs from the 10 monitored were found in these samples. However, only nonylphenol was detectable once the analysis value is 9.97±2.64 ng L^{-1}, above the LOD (7.17 ng L^{-1}) calculated for this compound.

As shown in Fig. 3e, related with chlorinated water, it is not possible the detection and quantification of any of the compounds under study. The analysis of just one sample is not representative of the matrix, so that monitoring should include a larger number of samples.

A global analysis of all results obtained by SPE-UPLC-ESI-MS/MS and comparing these values with LOD and LOQ calculated for each endocrine-disrupting compound allow some conclusions. For natural endocrine-disrupting compounds, such as estriol, 17-β-estradiol and estrone, related with groundwater catchment, the average concentration of estriol was 2.69±0.99 ng L^{-1} which is consistent with Hohenblum et al. (2004), who conclude that levels of natural estrogens in groundwater catchments investigated in Austria were below than 1 ng L^{-1} in most cases, whereas in France, they reached up to 3.5 ng L^{-1}. On this sampling point, estriol was the only natural hormone with concentration values that allow the monitoring of the presence of this EDC in this study. In reservoirs, four samples had values of estriol higher than the method LOD, corresponding to 9.5 % of the samples collected in this sampling point. Also, two samples in pumping stations had concentration values above LOD, corresponding to a total of 66.7 % of the collected samples. Concerning synthetic endocrine-disrupting compounds, such as ethinylestradiol, progesterone, mestranol, and diethylstilbestrol, they were only found in trace amounts. However, only mestranol had concentration values that allow some qualitative conclusions. For a total of 12 samples of groundwater catchments, one of the samples had a value higher than the method LOD and in reservoirs, and only two of samples had values above the LOD. None of samples allowed the quantification of EDCs due to the concentrations values being lower than LOQ.

Nonylphenol, an industrial product with endocrine-disrupting potential, was detected in 27 samples, ranging from 4.90 to 19.2 ng L^{-1}. This upper value corresponds to the pumping station, and it is close to the LOQ (21.5 ng L^{-1}) calculated for this compound which is in accordance with studies developed in Japan, Spain, Canada, the USA, and Germany that showed nonylphenol concentrations in river water samples,

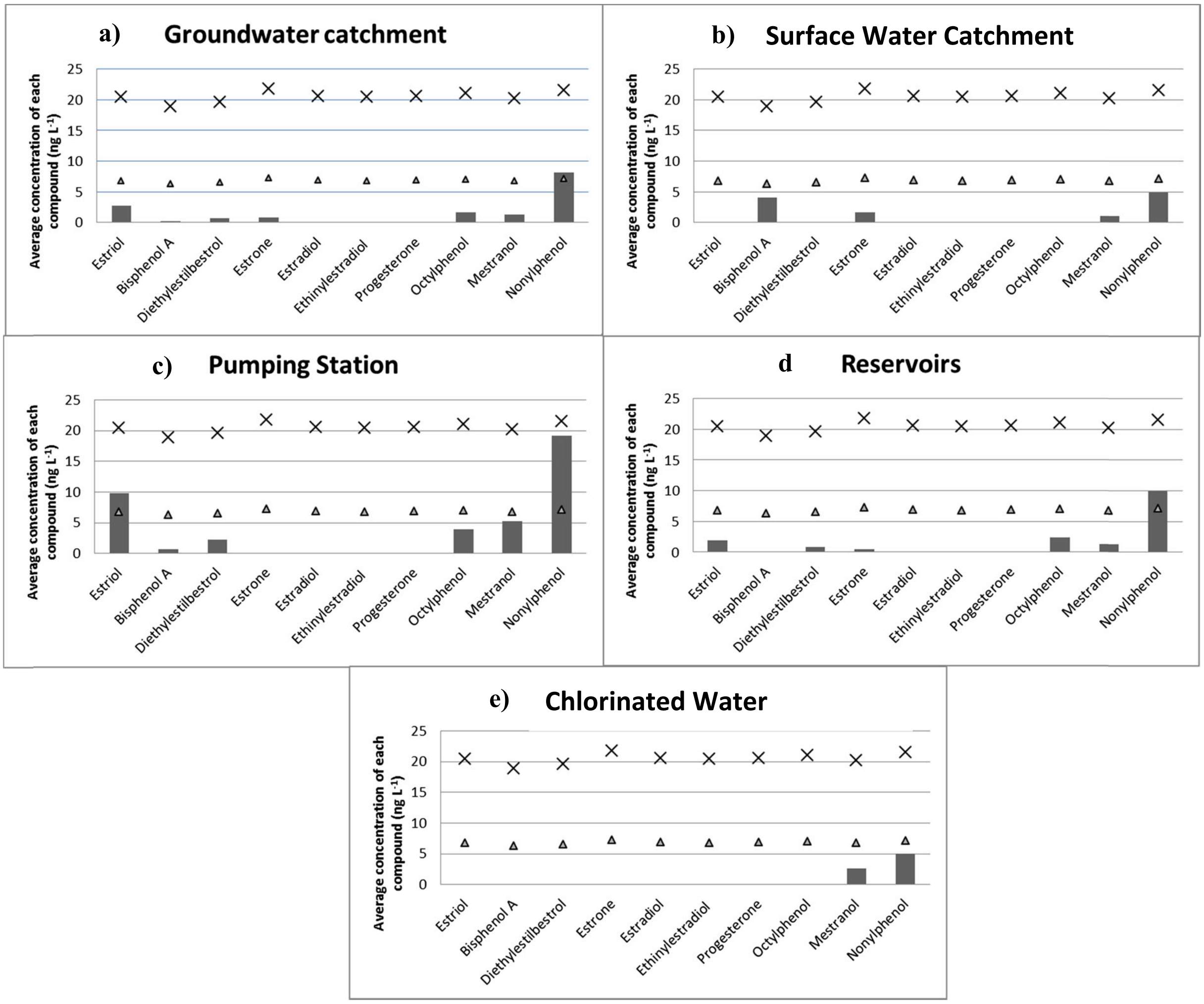

Fig. 3 Concentration of target EDCs in each sampling points: **a** groundwater catchment; **b** surface water catchment; **c** pumping station; **d** reservoirs; **e** chlorinated water. *Gray triangle* corresponds to LOD (ng L^{-1}); *multiplication symbol* corresponds to LOQ (ng L^{-1})

ranging from undetectable to 17.5 μg L^{-1} (Bennie et al. 1997; Kolpin et al. 2002; Cespedes et al. 2008). More recent studies conducted by Gorga et al. (2014) concluded that this EDC is found in lower concentrations in Spanish rivers which were in accordance with the results obtained in the present work.

In conclusion, the estrogenic risk to aquatic organisms and human health is low when comparing with the EQS for nonylphenol and other priority substances 0.30 and 0.10 μg L^{-1}, respectively (2013/39/EC Directive). Although, considering the unpredictable behavior characteristic of endocrine-disrupting compounds, it is important to take into account that are mixtures of sub-products that could influence the estrogenicity of the environmental matrix in study (Esteban et al. 2014; Gorga et al. 2014).

Conclusions

The main conclusions of this work, applying a SPE-UPLC-ESI-MS/MS method for the determination of 10 endocrine-disrupting compounds in 60 several samples collected along a water supply system: groundwater catchment, surface water catchment, pumping stations, reservoirs, and chlorinated water, were as follows:

- SPE-UPLC-ESI-MS/MS method has been used for detection and quantification because of its high sensitivity and opportunity of confirmation when two MRM transitions for each compound are recorded.
- Results from the monitoring performed confirmed the presence of some of the target compounds and

further toxicological studies should be performed. In fact, estriol, octylphenol, mestranol, and nonylphenol were detected in some samples but only estriol and nonylphenol had concentration levels that allowed its quantification. However, these EDC were found in concentration levels lower than the proposal value of 0.1 μg L^{-1}, established by the Drinking Water Directive for compounds without guideline values, taking into account the precautionary principle.

- In future, these studies should be routinely continued in order to prevent the increase of these target compounds concentration at the catchments areas and drinking water distribution system to guarantee the high quality level required by consumers each time more informed and aware of the risks for human health caused by the presence of the EDCs compounds emerging from different sources.

Compliance with ethical standards I testify on behalf of all co-authors that regarding our paper submitted to *Environmental Monitoring and Assessment*:

The manuscript has not been submitted to more than one journal for simultaneous consideration.

The manuscript has not been published previously (partly or in full), unless the new work concerns an expansion of previous work (please provide transparency on the re-use of material to avoid the hint of text-recycling (“self-plagiarism”)).

A single study is not split up into several parts to increase the quantity of submissions and submitted to various journals or to one journal over time (e.g., “salami-publishing”).

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Consent to submit has been received explicitly from all co-authors, as well as from the responsible authorities—tacitly or explicitly—at the institute/organization where the work has been carried out, before the work is submitted.

Authors whose names appear on the submission have contributed sufficiently to the scientific work and therefore share collective responsibility and accountability for the results.

References

Bennie, D. T., Sullivan, C. A., Lee, H. B., Peart, T. E., & Maguire, R. J. (1997). Occurrence of alkylphenols and alkylphenol mono- and diethoxylates in natural water of Laurentian Great Lakes basin and the upper St. Lawrence River. *Science of The Total Environment, 193*, 263–275.

Boles, T. H., & Wells, M. J. M. (2010). Analysis of amphetamine and methamphetamine as emerging pollutants in wastewater and wastewater-impacted streams. *Journal of Chromatography A, 1217*, 2561–2568.

Carvalho, A.R.M. (2012). Monitoring of endocrine disruptors in water supply and WWTP by SPE-UPLC-ESI-MS/MS. EPAL. Master Degree Thesis in Environmental Engineering. Technical University of Lisbon. http://hdl.handle.net/10400.5/5371.

Céspedes, R., Lacorte, S., Petrovic, M., & Barceló, D. (2008). Occurrence and fate of alkylphenols and alkylphenol ethoxylates in sewage treatment plants and impact on receiving waters along the Ter River (Catalonia, NE Spain). *Environmental Pollution, 153*, 384–392.

Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption. (1998). Official Journal L 330, pp 32–54 (ES, DA, DE, EL, EN, FR, IT, NL, PT, FI, SV).

Directive 2013/39/EU of the European Parliament and of the Council of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy (Text with EEA relevance). (2013). Official Journal L 226, pp 1–17 (BG, ES, CS, DA, DE, ET, EL, EN, FR, HR, IT, LV, LT, HU, MT, NL, PL, PT, RO, SK, SL, FI, SV).

Dolar, D., Gros, M., Rodriguez-Mozaz, S., Moreno, J., Comas, J., Rodriguez-Roda, I., & Barceló, D. (2012). Removal of emerging contaminants from municipal wastewater with an integrated membrane system, MBR-RO. *Journal of Hazard Materials, 15*(239–240), 64–9.

Esteban, S., Gorga, M., Petrovic, M., González-Alonso, S., Barceló, D., & Valcárcel, Y. (2014). Analysis and occurrence of endocrine-disrupting compounds and estrogenic activity in the surface waters of Central Spain. *Science of the Total Environment, 466–46*, 939–951.

Fredj, S. B., Nobbs, J., Tizaoui, C., & Monser, L. (2015). Removal of estrone (E1), 17β -estradiol (E2) and 17α-ethinylestradiol (EE2) from wastewater by liquid–liquid extraction. *Chemical Engineering Journal, 262*, 417–426.

Fuhrman, V. F., Tal, A., & Arnon, S. (2015). Why endocrine disrupting chemicals (EDCs) challenge traditional risk assessment and how to respond. *Journal of Hazardous Materials, 286*, 589–611.

Gorga, M., Insa, S., Petrovic, M., & Barceló, D. (2014). Occurrence and spatial distribution of EDCs and related compounds in waters and sediments of Iberian rivers. *Science of The Total Environment, 503–504*, 69–86.

Hohenblum, P., Gans, O., Moche, W., Scharf, S., & Lorbeer, G. (2004). Monitoring of selected estrogenic hormones and industrial chemicals in groundwaters and surface waters in Austria. *Science of The Total Environment, 333*, 185–193.

Kasprzyk-Hordern, B., Dinsdale, R. M., & Guwy, A. J. (2008). The occurrence of pharmaceuticals, personal care products, endocrine disruptors and ilicit drugs in surface water in South Wales, UK. *Water Research, 42*(13), 3498–3518.

Kolpin, D. W., Furlong, E. T., Meyer, M. T., Thurman, E. M., Zaugg, S. D., Barber, L. B., Buxton, H. T. (2002). Pharmaceuticals, hormones and other organic wastewater contaminants in U.S. streams 1999-2000: a national reconnaissance. *Environmental Science Technology, 36*(6), 1020–1211.

Kosjek, T., Heath, E., & Krbavčič, A. (2005). Determination of non-steroidal anti-inflammatory drug (NSAIDs) residues in water samples. *Environment International, 31*, 679–685.

López-Roldán, R., Alda, M. L., Gros, M., Petrovic, M., Martin-Alonso, J., & Barceló, D. (2010). Advanced monitoring of pharmaceuticals and estrogens in the Llobregat River basin (Spain) by liquid chromatography-triple quadrupole-tandem mass spectrometry in combination with ultra performance liquid chromatography-time of flight-mass spectrometry. *Chemosphere, 80*, 1337–1344.

ME 72 (2010). Determinação quantitativa de desreguladores endócrinos - Método de Cromatografia de Ultra Resolução associada à espectrometria de massas. Internal method from EPAL.

Penetra, A., Cardoso, V., Ferreira, E., & Benoliel, M. (2010). Solid-phase extraction and gas chromatography-tandem mass spectrometry method for the simultaneous determination of several pesticides in water. *Water Science and Technology, 62*(3), 667–675.

San Filippo, K., Pinto, B., Colombini, M. P., Bartolucci, U., & Reali, D. (2010). Determination of trace endocrine disruptors in ultrapure water for laboratory use by the yeast estrogen screen (YES) and chemical analysis (GC/MS). *Journal of Chromatography B, 878*, 1190–4.

Sebők, Á., Vasanits-Zsigrai, A., Helenkár, A., Záray, G., & Molnár-Perl, I. (2009). Multiresidue analysis of pollutants as their trimethylsilyl derivatives, by gas chromatography–mass spectrometry. *Journal Chromatography A, 1216*, 2288–2301.

Stanford, B. D., & Weinberg, H. S. (2007). Isotope dilution for quantification of steroid estrogens and nonylphenols by gas chromatographic with tandem mass spectrometry in septic, soil and groundwater matrices. *Journal of Chromatography A*, 1176, 26–36.