



# Evaluation, comparison and combination of molecularly imprinted polymer solid phase extraction and classical solid phase extraction for the preconcentration of endocrine disrupting chemicals from representative whole water samples

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## ABSTRACT

Estrogens are endocrine disrupting chemicals and of high concerns due to demonstrated harmful effects on the environment and low effect levels. For monitoring and risk assessment, several estrogens were included in the "watch list" of the EU Water Framework Directive which sets very low environmental quality standard (EQS) levels for Estrone (E1) and 17 $\beta$ -Estradiol (E2) of 0.4 ng L<sup>-1</sup> and for 17 $\alpha$ -Ethinylestradiol (EE2) of 0.035 ng L<sup>-1</sup> requiring sensitive detection methods, as well as extensive sample preparation. A sensitive, derivatization-free, isotope dilution calibration HPLC-MS/MS method for a panel of 5 selected estrogens (including the 3 estrogens of the EU WFD watchlist), and a procedure for the reproducible preparation of a representative whole water matrix including mineral water, humic acids and solid particulate matter are presented. These are used in a diligent comparison of classical solid phase extraction (SPE) on hydrophilic-lipophilic balanced (HLB) phase to SPE on an estrogen-specific molecularly imprinted polymer phase (MISPE) for ultra-trace levels of the analytes (1–10 ng L<sup>-1</sup>). Additionally, a two-step procedure combining HLB SPE disks followed by MISPE is evaluated. The two-step procedure provides superior enrichment, matrix removal and sample throughput while maintaining comparable recovery rates to simple cartridge SPE. Estimated method quantification limits (MQLs) range from 0.109–0.184 ng L<sup>-1</sup> and thus meet EQS-levels for E1 and E2, but not EE2. The representative whole water matrix provides a reproducible comparison of sample preparation methods and lays the foundation for a certified reference material for estrogen analysis. The presented method will serve as the basis for an extended validation study to assess its use for estrogen monitoring in the environment.

## Introduction

Estrogens have been discussed as environmental pollutants since the early 1990s [1]. Estrogens are endocrine disrupting chemicals (EDCs) with very low effective concentrations. Their potential harmful effects have long been known but have since been observed in the environment as well [2–7]. In wastewater, but also in surface waters and drinking water, estrogens occur in sometimes alarmingly high concentrations [8–16]. Three particularly relevant estrogens, estrone (E1), 17 $\beta$ -estradiol (E2) and 17 $\alpha$ -ethinylestradiol (EE2), were therefore included as priority substances in the EU Water Framework Directive (EU WFD) at the turn of the millennium [17]. The EU also implemented environmental quality standards (EQS) for the concentration of these estrogens

in surface waters. Due to small predicted no-effect concentrations [18], these are in a very low range of 0.4 ng L<sup>-1</sup> for E2 [19] and 0.035 ng L<sup>-1</sup> for EE2 [19]. These low concentrations are challenging even for state-of-the-art methods and equipment, although the determination of estrogens in environmental samples is well established [2,8–12,16,20,21].

Despite the plethora of methods for quantifying estrogens in environmental matrices, few achieve the low detection limits required by EU WFD standards – especially not for EE2 [22,23]. Comprehensive sample preparation is essential in this regard. Filtration and multiple extraction steps are common [24–28]. Methods have been published for up to 10 L of sample [29]. However, large sample volumes usually lower throughput which is important for efficient monitoring programs.

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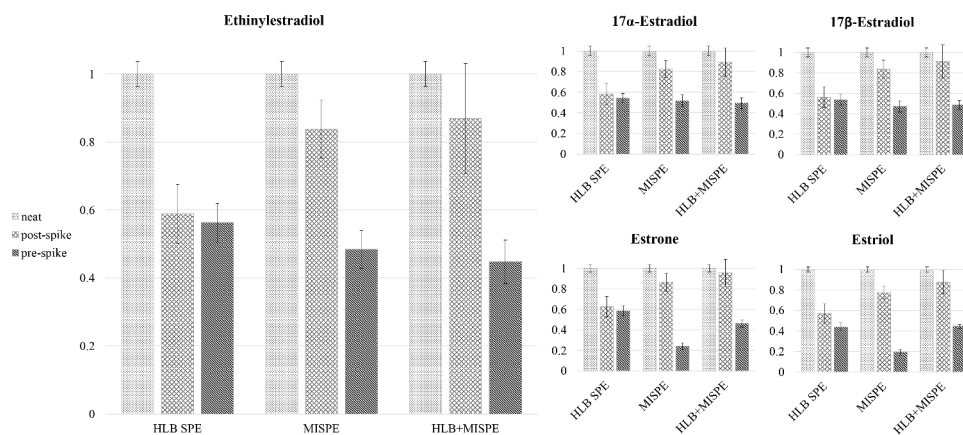
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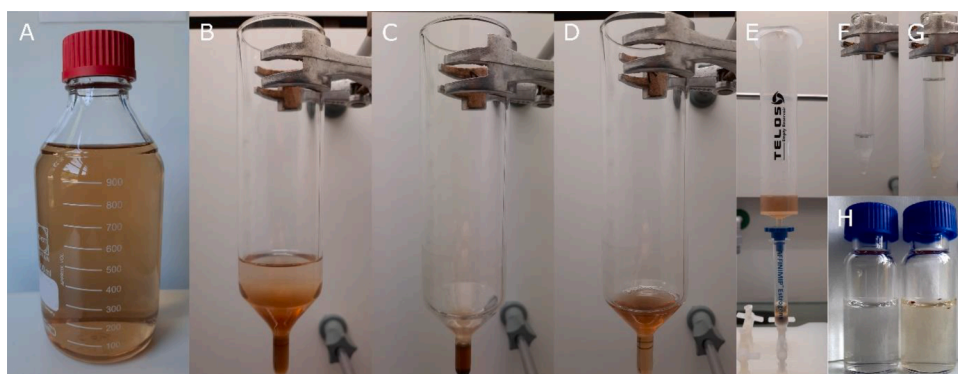
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**Fig. 1.** Result of the three different sample preparation procedures. Shown are measured final concentrations of the respective analyte normalized to the neat standard. Dotted bars show the neat standard, rhombic bars the post-spike and hatched bars the pre-spike sample. Normalized pre-spike concentration is equal to absolute recovery. The difference between post-spike and neat standard corresponds to the matrix effect and the difference between post-spike and pre-spike to loss of analyte. Error bars are standard deviations of triplicate samples with three injections. Note that the standard deviation for the post-spike samples is higher because the analytes were added volumetrically for practical reasons.



**Fig. 2.** Representative whole water matrix and SPE procedure. (A) A representative 1 L sample of the representative whole water matrix (B) 50 mL of SPE disk eluate of a 1 L sample of representative whole water matrix (C) SPE disk eluate after evaporation to 1 mL (D) SPE disk eluate diluted in 10 mL of H<sub>2</sub>O (E) Diluted SPE disk eluate applied to MISPE column. (F) 3 mL of MISPE eluate of a 0.1 L sample of representative whole water matrix (G) HLB-SPE eluate of a 0.1 L sample of representative whole water matrix (H) 1 mL final sample after MISPE (left) and HLB-SPE (right), respectively. Note that eluate and final sample after MISPE and combined SPE disk and MISPE procedure are visually indistinguishable from each other, which is why only one representative sample is shown here.

**Table 1**

Instrument detection and quantification limits (IDL and IQL), relative standard deviation (RSD) of the calibration and linearity R calculated according to DIN 32645 for  $N = 11$  standard levels with  $n = 3$  injections (significance level  $\alpha = 0.01$  and coverage factor  $k = 3$ ). Estimated method detection limit (MDL) and quantification limit (MQL) are based on IDL and IQL factoring in recovery and preconcentration of the two-step process.

Analyte	IDL [ng L <sup>-1</sup> ]	IQL [ng L <sup>-1</sup> ]	RSD [%]	R	Estimated MDL [ng L <sup>-1</sup> ]	Estimated MQL [ng L <sup>-1</sup> ]
E1	15	51	1.2	0.9998	0.033	0.109
E2	16	53	1.6	0.9996	0.033	0.109
E2 $\alpha$	28	91	2.0	0.9993	0.058	0.184
E3	24	77	2.1	0.9992	0.054	0.172
EE2	26	82	2.3	0.9991	0.058	0.184

Selective enrichment is of particular importance here to minimize the co-purification of interfering substances. Ubiquitous dissolved organic carbon (DOC) like humic acids may be concentrated along with the

**Table 2**

Relative recoveries in the pre-spiked samples for each SPE procedure, i.e., the quotient of the area-to-concentration ratio of the native analytes and respective internal standards. Standard deviations are calculated for triplicate samples with three injections.

	HLB		MIP		HLB+MIP	
	Relative recovery [%]	Standard deviation [%]	Relative recovery [%]	Standard deviation [%]	Relative recovery [%]	Standard deviation [%]
Estrone	0.9700	0.0025	0.9254	0.0105	0.9620	0.0030
Estradiol	1.0002	0.0001	1.0002	0.0001	1.0001	0.0001
17 $\alpha$ -Estradiol	1.0009	0.0002	1.0007	0.0004	1.0014	0.0006
Estriol	0.9965	0.0009	0.9916	0.0021	0.9960	0.0007
Ethinylestradiol	1.0017	0.0005	1.0021	0.0006	1.0012	0.0004

17 $\alpha$ -Estradiol (E2 $\alpha$ ). E3 is one of the three major human estrogens, together with E1 and E2, which constitutes the major free estrogen in human urine due to its increased water solubility. Besides its pharmaceutical use as a hair growth promoter, E2 $\alpha$  is the primary estrogen in cattle, making it a severe environmental concern in dairy wastewater and manure [39]. More recently its human excretion has also received scrutiny [40]. Due to their physicochemical similarity, the resolution of the two isomers of E2 is also a useful benchmark for the efficiency of a chromatographic separation.

## Methods

### Materials and chemicals

For the preparation of the representative whole water matrix, a commercially available non-sparkling mineral water with known inorganic composition and distinct pH value of 7.3 was used. Humic acids (sodium salt, technical grade, Sigma-Aldrich Pty Ltd, an affiliate of Merck KGaA, Darmstadt, Germany) and fresh-water sediment (certified reference material CRM016, Sigma-Aldrich Pty Ltd, an affiliate of Merck KGaA, Darmstadt, Germany) were purchased as dry solids. For the filtration of the humic acid stock solution, 0.45  $\mu$ m polyethersulfone (PES) filters from Sarstedt (Nuembrecht, Germany) were used.

Oasis® HLB cartridges (6 mL, 150 mg) from Waters (Milford, MA, USA), Affinimip® SPE Estrogens cartridges (3 mL, 100 mg) from Affinisep (Le Houlme, France) and Atlantic® HLB-H Disks (47 mm) from Biotage (Uppsala, Sweden) were applied for extraction, preconcentration and purification, respectively.

Ultrapure water was generated with an ELGA Purelab® flex (Veolia Water Technologies, Paris, France) water purifier. ULC/MS grade solvents methanol and acetonitrile were obtained from Biosolve B.V. (Valkenswaard, Netherlands). Ammonium fluoride ( $\geq 99.99$  % trace metals basis) was purchased from Sigma-Aldrich (St. Louis, MO, USA).

### Measurement standards

Standards solutions for E1, E2 $\alpha$ , E2, EE2 and E3 were prepared from ISO 17034 reference materials (LGC standards Ltd., Teddington, UK). Isotopically labelled internal standards 3,4-13C2-17 $\beta$ -Estradiol (LGC standards Ltd., Teddington, UK), as well as 17,17-13C2-17 $\alpha$ -Ethinylestradiol, 2,4-D2-17 $\alpha$ -Estradiol, 2,4-D2-Estriol and 2,4-D2-Estrone (CDN Isotopes, Pointe-Claire, Canada) were used for stable isotope dilution. Stock solutions of these compounds were prepared and diluted gravimetrically. From these, a calibration curve of 0.1 to 1 ng mL<sup>-1</sup> was generated with internal standards at 0.5 ng L<sup>-1</sup>. A mixture of all 5 analytes and the respective internal standards of 10 ng mL<sup>-1</sup> each in methanol was used for spiking the samples.

### Representative whole water matrix

For reproducible adjustment of the DOC content, a stock solution of humic acids of defined concentration in mineral water was developed. To prepare the humic acids stock solution, 4 g of humic acids are suspended in 1 L of mineral water and sonicated for 1 h at room temperature in an ultrasonic bath. The solution is then sedimented overnight at 4°C in a refrigerator. The next day, the solution is decanted and centrifuged (4000 x g, 60 min). A deep black suspension is obtained. This stock solution remains stable without precipitating upon further storage at 4°C. An aliquot of the stock solution is filtered with a syringe filter (0.45  $\mu$ m) and the DOC content is determined as non-purgeable organic carbon (NPOC) on a TOC-L series TOC analyser (Shimadzu, Kyoto, Japan). The SPM content is adjusted by adding a weighed amount of freshwater sediment reference material to the water matrix.

### Solid phase extraction

For HLB-SPE and MISPE (supplementary Table 3), 0.1 L representative whole water matrix samples were used. Pre-spike samples were generated by sinking a vial containing 100  $\mu$ L of a mixture containing 10 ng mL<sup>-1</sup> of all analytes and the appropriate internal standards. The volume of the vial content is controlled gravimetrically. The samples were put on a horizontal shaker at 120 strokes per min for 30 min to equilibrate. For post-spike samples, the same amount was added to the eluate by pipetting. The solvent was evaporated under N<sub>2</sub> in a Turbo-Vap® II (Biotage, Uppsala, Sweden) evaporator to dryness and was reconstituted in 1 mL 20% acetonitrile. Separate samples were spiked before and after SPE processing to estimate matrix effects. For comparison, 1 mL clean standards were also prepared in triplicate to 1 ng mL<sup>-1</sup> in 20% acetonitrile.

For the two-step procedure 1 L of sample was prepared and preconcentrated using HLB SPE disks (supplementary Table 3). Pre- and Post-spike samples were spiked with 100  $\mu$ L of estrogen solution as described above. The extract was reduced to 1 mL in a TurboVap® LV (Biotage, Uppsala, Sweden) evaporator with volume sensor (at 1 mL). Then, 10 mL of ultrapure water were added, and the diluted sample was purified by the same procedure as the MISPE-only samples. Post-spike samples were only spiked after the second step.

All samples were prepared and worked up in triplicate. In addition, the dry weight after SPE was determined for each of the sample preparation methods with representative samples without estrogens to get a quantitative estimate of matrix removal.

### HPLC-MS/MS

Quantification was achieved on an Agilent 1260 Infinity II HPLC system coupled to an AB Sciex QTRAP® 6500 triple-quadrupole mass spectrometer equipped with an electrospray ion source (ESI) in negative ionization mode. An Agilent ZORBAX SB-Phenyl (80 Å, 1.8  $\mu$ m, 4.6 x 50 mm, 600 bar) column was used for separation. The mobile phase consisted of ultrapure water containing 0.25 mM ammonium fluoride (eluent A) and methanol (eluent B). Isocratic elution was performed at 60 % B for 15 min, washing for 10 min and re-equilibrating for another 10 min. The flowrate was 200  $\mu$ L min<sup>-1</sup>. MS-parameters and MRM transitions can be found in the supplementary material.

Isotope dilution calibration with an internal standard level of 500 ng L<sup>-1</sup> was used for all standards and samples to determine relative recoveries independent of the preconcentration procedure. Calibration curves can be found in the supplementary material. All samples and standards were injected three times and measured in random order. Blank samples were injected between each measurement to preclude carry-over.

In addition, the HPLC-MS/MS method was calibrated according to DIN 32645 [41] (data not shown) to obtain an estimate for the instrument detection and quantification limits (IDL and IQL). Estimates were then calculated for method detection and quantification limits (MDL and MQL) from the instrument limits factoring in the recovery and preconcentration of the two-step procedure according to

$$MDL \text{ or } MQL = \frac{IDL \text{ or } IQL}{\text{concentration factor} \cdot \text{recovery}}$$

with a concentration factor of one thousand (1 L to 1 mL) and the recovery of the respective analyte. This operates under the assumption that the matrix is quantitatively removed so that instrument performance is not affected by the sample.

## Results

A sensitive HPLC-MS/MS method for the quantification of the analyte panel in the low ng L<sup>-1</sup> range without the need for derivatisation

was developed and optimized. Linearity is very high with and without isotope dilution calibration and baseline separation of the critical pair E2 and E2 $\alpha$  was achieved with isocratic elution at room temperature.

The estrogen concentrations of 0.1 L (for HLB and MISPE) and 1 L (for the two-step process) representative whole water samples spiked with the five analytes at a concentration of 1 ng L<sup>-1</sup> were determined by LC-MS/MS. Fig. 1 shows, for all analytes, the concentrations of the samples spiked with estrogens before (shaded) and after (diamond) purification compared to the pure standard (dotted) with the respective sample preparation procedures. Absolute recovery was 20 to 60 % for all analytes. The values for estriol are somewhat lower due to its higher polarity. Although different sorption characteristics have been published for the two estradiol isomers [42], they and EE2 were found to behave similarly within the measurement error uncertainty. E1 is retained much more poorly in MISPE than E2.

The composition of the commercial MIP is not disclosed by the manufacturer. Although a variety of different functional monomers are available, specificity is most often conferred by hydrogen bonding [43]. The template is likely E2, as it is the most used template in the literature for Estrogen MIPs [44]. Therefore, the best recoveries are expected for E2, while the additional hydroxyl group of E3 might affect optimal binding. The same is true for the carbonyl group of E1, which cannot act as a hydrogen bond donor. In contrast, EE2 and E2 $\alpha$  provide the same functionality as E2 and only differ sterically, which is expected to have a smaller influence on the binding. Other functional monomers mediating ionic, or pi-pi interactions would interact with the phenol moiety and therefore would not cause discrimination between analytes. Hydrophobic interactions would favour E2, E2 $\alpha$  and EE2 over E1 and E3 as the latter are more polar.

To further confirm the matrix removal capability of the MISPE the dry weight after SPE was determined to quantify DOC removal. The result was 50 % and 70 % removal for the HLB cartridge and HLB SPE disk, respectively. For the MISPE and the two-step process, the dry weight was below the error of the fine balance ( $\pm 0.01$  mg, corresponding to 1.43 % of the sample DOC). In Fig. 2, the co-purified DOC after HLB disk and cartridge SPE is clearly visible as a yellowish to deep brown discoloration of the respective eluate. The final sample of the HLB-SPE (Fig. 2 H) retains a yellowish tint and small particles of aggregated humic acids are visible on the surface, whereas samples were always perfectly clear after the MISPE and the two-step procedure.

The estimated MQL for the two-step process is in the lower pg L<sup>-1</sup> range (Table 1). While absolute recoveries were similar for all analytes the response factor for EE2 was consistently the lowest of the analyte panel. Relative recoveries were very close to one hundred percent for all samples with very small standard deviations (Table 2). E1 shows a slight deviation.

## Discussion

While the HLB sorbent retains the analyte almost quantitatively, co-elution of matrix components leads to significant matrix effects that reduce measurement sensitivity and thus lower absolute recoveries. By contrast, the MIP sorbent was far superior in terms of matrix removal, but a considerable portion of analytes was lost. Overall, this makes performance of the two sorbents comparable, but for E1 and E3, the classic HLB SPE surpasses the MISPE. However, a combined method using an HLB step followed by MISPE for matrix removal proved to be a good compromise, offering decent recovery at very low matrix load. It was also found that pre-purification increases the efficiency of MISPE, with the 2-step procedure yielding twice as high recoveries for E1 and E3 as the MISPE alone. Although ten times the sample volume was used for the evaluation of the two-step procedure, performance was comparable to the other two methods. In particular, the matrix effect becomes negligible within measurement accuracy.

A reduced matrix load not only benefits sensitivity, but also extends the life of the HPLC and MS instrument components. In addition, if a

derivatization step is required for detection, a substantially smaller amount of reagent is consumed, reducing cost and waste. This is particularly important for gas chromatography (GC), which is still the most common analytical method in routine laboratories, as estrogens are not volatile enough for GC. This makes the sample preparation method highly suitable for routine analysis.

The use of disk SPE allows for higher flow rates, so the total sample preparation time of the two-step procedure barely exceeds that of a standard cartridge SPE. The SPE disk also allows higher sample volumes, which directly increases the detection limit of the method. In addition, SPE disks allow whole water analysis without the need for filtration while meeting EU WFD requirements. For this application, the two-step procedure can be recommended.

With the representative whole water matrix, it was possible to reduce sample variability to a minimum and achieve a rigorous comparison of the different procedures. The detrimental effect on MISPE recoveries illustrates, that the matrix is by no means oversimplified. This constitutes a reasonable basis for a future application as a certified reference material which is urgently needed for estrogen monitoring under EU WFD requirements.

The HPLC-MS/MS method is particularly suitable to routine analysis as it does not require derivatization of the analytes, reducing workload. Isotope dilution calibration compensates any losses during sample preparation formidably. Estimates for the MQL of the two-step process with HPLC-MS/MS detection do meet EU WFD requirements for E1 and E2, but not EE2 (184 pg L<sup>-1</sup> instead of 35 pg L<sup>-1</sup>). However, due to efficient matrix removal, little loss of sensitivity is expected if the sample, final, or injection volume of the method would be increased. This would not have been appropriate for the present study, as the robustness of the method was more crucial than its sensitivity for obtaining reliable results. A modified method based on the results of this work is currently being validated according to XP CEN/TS 16800 [45] for use within EU WFD monitoring programs as part of the EU project 18NMR01. The results are expected to be published shortly.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data Availability

Data will be made available on request.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.talo.2022.100163.

## Literature

- [1] L.S. Shore, M. Gurevitz, M. Shemesh, Estrogen as an environmental pollutant, *Bull. Environ. Contam. Toxicol.* 51 (3) (1993) 361–366.
- [2] L. Barreiros, J.F. Queiroz, L.M. Magalhães, A.M.T. Silva, M.A. Segundo, Analysis of 17- $\beta$ -estradiol and 17- $\alpha$ -ethinylestradiol in biological and environmental matrices — A review, *Microchem. J.* 126 (2016) 243–262.
- [3] M. Clemons, P. Goss, Estrogen and the risk of breast cancer, *N. Engl. J. Med.* 344 (4) (2001) 276–285.

- [4] K.A. Kidd, P.J. Blanchfield, K.H. Mills, V.P. Palace, R.E. Evans, J.M. Lazorchak, R. W. Flick, Collapse of a fish population after exposure to a synthetic estrogen, *Proc. Natl Acad. Sci.* 104 (21) (2007) 8897–8901.
- [5] M. Giulivo, M. Lopez de Alda, E. Capri, D. Barceló, Human exposure to endocrine disrupting compounds: Their role in reproductive systems, metabolic syndrome and breast cancer. A review, *Environ. Res.* 151 (2016) 251–264.
- [6] J.P. Laurenson, R.A. Bloom, S. Page, N. Sadrieh, Ethinyl estradiol and other human pharmaceutical estrogens in the aquatic environment: a review of recent risk assessment data, *AAPS J.* 16 (2) (2014) 299–310.
- [7] M. Adeel, X. Song, Y. Wang, D. Francis, Y. Yang, Environmental impact of estrogens on human, animal and plant life: a critical review, *Environ. Int.* 99 (2017) 107–119.
- [8] C. Miège, V. Gabet, M. Coquery, S. Karolak, M.L. Jugan, L. Oziol, Y. Lévi, M. Chevreuil, Evaluation of estrogenic disrupting potency in aquatic environments and urban wastewaters by combining chemical and biological analysis, *Trends Anal. Chem.* 28 (2009) 186–195.
- [9] G. Streck, Chemical and biological analysis of estrogenic, progestagenic and androgenic steroids in the environment, *TrAC Trends Anal. Chem.* 28 (6) (2009) 635–652.
- [10] H. Hamid, C. Eskicioglu, Fate of estrogenic hormones in wastewater and sludge treatment: a review of properties and analytical detection techniques in sludge matrix, *Water Res.* 46 (18) (2012) 5813–5833.
- [11] R.L. Gomes, M.D. Scrimshaw, J.N. Lester, Determination of endocrine disruptors in sewage treatment and receiving waters, *Trends Anal. Chem.* 22 (10) (2003) 697–707.
- [12] V. Gabet, C. Miège, P. Bados, M. Coquery, Analysis of estrogens in environmental matrices, *Trends Anal. Chem.* 26 (11) (2007) 1113–1131.
- [13] S. Esteban, M. Gorga, M. Petrovic, S. Gonzalez-Alonso, D. Barceló, Y. Valcarcel, Analysis and occurrence of endocrine-disrupting compounds and estrogenic activity in the surface waters of Central Spain, *Sci. Total Environ.* 466–467 (2014) 939–951.
- [14] C.G. Campbell, S.E. Borglin, F.B. Green, A. Grayson, E. Wozei, W.T. Stringfellow, Biologically directed environmental monitoring, fate, and transport of estrogenic endocrine disrupting compounds in water: a review, *Chemosphere* 65 (8) (2006) 1265–1280.
- [15] D. Álvarez-Muñoz, S. Rodríguez-Mozaz, A.L. Maulvault, A. Tediosi, M. Fernández-Tejedor, F. Van den Heuvel, M. Kotterman, A. Marques, D. Barceló, Occurrence of pharmaceuticals and endocrine disrupting compounds in macroalgae, bivalves, and fish from coastal areas in Europe, *Environ. Res.* 143 (Pt B) (2015) 56–64.
- [16] K. Kozłowska-Tylingo, J. Namieśnik, T. Górecki, Determination of estrogenic endocrine disruptors in environmental samples—a review of chromatographic methods, *Crit. Rev. Anal. Chem.* 40 (3) (2010) 194–201.
- [17] Directive 2000/60/EC of the European parliament and of the council of 23 October 2000 establishing a framework for Community action in the field of water policy, *Off. J. L* 327 (2000) 0001–0073.
- [18] D.J. Caldwell, F. Mastrocco, P.D. Anderson, R. Länge, J.P. Sumpter, Predicted-no-effect concentrations for the steroid estrogens estrone, 17 $\beta$ -estradiol, estriol, and 17 $\alpha$ -ethinylestradiol, *Environ. Toxicol. Chem.* 31 (6) (2012) 1396–1406.
- [19] Commission implementing decision (EU) 2015/495 of 20 March 2015 establishing a watch list of substances for Union-wide monitoring in the field of water policy pursuant to Directive 2008/105/EC of the European Parliament and of the Council, *Off. J. L* 78 (2015) 40–42.
- [20] Z.H. Liu, G.N. Lu, H. Yin, Z. Dang, H. Littler, Y. Liu, Sample-preparation methods for direct and indirect analysis of natural estrogens, *TrAC Trends Anal. Chem.* 64 (2015) 149–164.
- [21] T.Y. Fang, S.M. Praveena, C. deBurbure, A.Z. Aris, S.N.S. Ismail, I. Rasdi, Analytical techniques for steroid estrogens in water samples - a review, *Chemosphere* 165 (2016) 358–368.
- [22] R. Loos, Analytical Methods for Possible WFD 1st Watch List Substances: Directive 2008/105/EC, as Amended by Directive 2013/39/EU in the Field of Water Policy, Joint Research Centre, Institute for Environment and Sustainability, 2016. Publications Office.
- [23] E.J. Tiedeken, A. Tahar, B. McHugh, N.J. Rowan, Monitoring, sources, receptors, and control measures for three European Union watch list substances of emerging concern in receiving waters – A 20year systematic review, *Sci. Total Environ.* 574 (2017) 1140–1163.
- [24] E. Heath, T. Kosjek, H.R. Andersen, H.C. Holten Lützhøft, M. Adolfsen Erici, M. Coquery, R.A. Düring, O. Gans, C. Guignard, P. Karlsson, F. Manciot, Z. Moldovan, D. Patureau, L. Cruceru, F. Sacher, A. Ledin, Inter-laboratory exercise on steroid estrogens in aqueous samples, *Environ. Pollut.* 158 (3) (2010) 658–662.
- [25] S. Barreca, M. Busetto, L. Colzani, L. Clerici, D. Daverio, P. Dellavedova, S. Balzamo, E. Calabretta, V. Ubaldi, Determination of estrogenic endocrine disruptors in water at sub-ng L–1 levels in compliance with Decision 2015/495/EU using offline-online solid phase extraction concentration coupled with high performance liquid chromatography-tandem mass spectrometry, *Microchem. J.* 147 (2019) 1186–1191.
- [26] A. Rubirola, M.R. Boleda, M.T. Galceran, Multiresidue analysis of 24 Water Framework Directive priority substances by on-line solid phase extraction-liquid chromatography tandem mass spectrometry in environmental waters, *J. Chromatogr. A* 1493 (2017) 64–75.
- [27] A. Glineur, K. Nott, P. Carboneille, S. Ronkart, G. Purcaro, Development and validation of a method for determining estrogenic compounds in surface water at the ultra-trace level required by the EU Water Framework Directive watch list, *J. Chromatogr. A* 1624 (2020), 461242.
- [28] M.V. Salvia, E. Vulliet, L. Wiest, R. Baudot, C. Cren-Olivé, Development of a multi-residue method using acetonitrile-based extraction followed by liquid chromatography-tandem mass spectrometry for the analysis of steroids and veterinary and human drugs at trace levels in soil, *J. Chromatogr. A* 1245 (2012) 122–133.
- [29] M. Česen, E. Heath, Disk-based solid phase extraction for the determination of diclofenac and steroidal estrogens E1, E2 and EE2 listed in the WFD watch list by GC-MS, *Sci. Total Environ.* 590–591 (2017) 832–837.
- [30] J.P. Antignac, K. de Wasch, F. Monteau, H. De Brabander, F. Andre, B. Le Bizec, The ion suppression phenomenon in liquid chromatography–mass spectrometry and its consequences in the field of residue analysis, *Anal. Chim. Acta* 529 (1) (2005) 129–136.
- [31] D. Zacs, I. Perkons, V. Bartkevics, Determination of steroidal oestrogens in tap water samples using solid-phase extraction on a molecularly imprinted polymer sorbent and quantification with gas chromatography-mass spectrometry (GC-MS), *Environ. Monit. Assess.* 188 (7) (2016) 433.
- [32] P. Lucci, O. Núñez, M.T. Galceran, Solid-phase extraction using molecularly imprinted polymer for selective extraction of natural and synthetic estrogens from aqueous samples, *J. Chromatogr. A* 1218 (30) (2011) 4828–4833.
- [33] M. Fiori, C. Civitareale, S. Mirante, E. Magarò, G. Brambilla, Evaluation of two different clean-up steps, to minimise ion suppression phenomena in ion trap liquid chromatography–tandem mass spectrometry for the multi-residue analysis of beta agonists in calves urine, *Anal. Chim. Acta* 529 (1) (2005) 207–210.
- [34] M. Nie, C. Yan, W. Dong, M. Liu, J. Zhou, Y. Yang, Occurrence, distribution and risk assessment of estrogens in surface water, suspended particulate matter, and sediments of the Yangtze Estuary, *Chemosphere* 127 (2015) 109–116.
- [35] S. Grund, E. Higley, R. Schönenberger, M.J. Suter, J.P. Giesy, T. Braunbeck, M. Hecker, H. Hollert, The endocrine disrupting potential of sediments from the Upper Danube River (Germany) as revealed by *in vitro* bioassays and chemical analysis, *Environ. Sci. Pollut. Res. Int.* 18 (3) (2011) 446–460.
- [36] Y. Wang, Q. Wang, L. Hu, G. Lu, Y. Li, Occurrence of estrogens in water, sediment and biota and their ecological risk in Northern Taihu Lake in China, *Environ. Geochem. Health* 37 (1) (2015) 147–156.
- [37] C.W. Walker, J.E. Watson, Adsorption of estrogens on laboratory materials and filters during sample preparation, *J. Environ. Qual.* 39 (2) (2010) 744–748.
- [38] G. Andaluri, R.P.S. Suri, K. Graham, Steroid hormones in environmental matrices: extraction method comparison, *Environ. Monit. Assess.* 189 (12) (2017) 626.
- [39] T.A. Hanselman, D.A. Graetz, A.C. Wilkie, Manure-borne estrogens as potential environmental contaminants: a review, *Environ. Sci. Technol.* 37 (24) (2003) 5471–5478.
- [40] Z. Tang, Z.H. Liu, H. Wang, Z. Dang, 17 $\alpha$ -Estradiol, an ignored endogenous natural estrogen in human: updated estrogen metabolism pathways and its environmental risk analysis, *Sci. Total Environ.* 829 (2022), 154693.
- [41] DIN 32645, Chemical Analysis - Decision Limit, Detection Limit and Determination Limit Under Repeatability Conditions - TERMS, Method, Evaluation, Beuth Verlag, Berlin, 2008. -11.
- [42] M.L. Mashtare, B. Khan, L.S. Lee, Evaluating stereoselective sorption by soils of 17 $\alpha$ -estradiol and 17 $\beta$ -estradiol, *Chemosphere* 82 (6) (2011) 847–852.
- [43] K. Haupt, P.X. Medina Rangel, B.T.S. Bui, Molecularly Imprinted polymers: antibody mimics for bioimaging and therapy, *Chem. Rev.* 120 (17) (2020) 9554–9582.
- [44] M. Doué, E. Bichon, G. Dervilly-Pinel, V. Pichon, F. Chapuis-Hugon, E. Lesellier, C. West, F. Monteau, B. Le Bizec, Molecularly imprinted polymer applied to the selective isolation of urinary steroid hormones: an efficient tool in the control of natural steroid hormones abuse in cattle, *J. Chromatogr. A* 1270 (2012) 51–61.
- [45] XP CEN/TS 16800, Guideline for the validation of physico-chemical analytical methods. ICS 13.060.50, (2021).