Anal Bioanal Chem (2005) 381: 1199–1205
DOI 10.1007/s00216-005-3055-1

ORIGINAL PAPER

Pierre Labadie · Hélène Budzinski

Development of an analytical procedure for determination of selected estrogens and progestagens in water samples

Received: 23 August 2004 / Revised: 12 December 2004 / Accepted: 14 December 2004 / Published online: 3 March 2005

Abstract An analytical procedure has been developed for determination of eight selected natural and synthetic hormonal steroids in surface water and in effluent samples. Several methodological points have been investigated and are discussed; they include the choice of the solid-phase extraction sorbent, the influence of flow rate on recovery, the breakthrough volume for a given sorbent (Env+ and Oasis HLB), sample clean up, and sample storage. As regards the latter point, it was found that when no preservative was added to effluent from a sewage-treatment plant, severe loss of steroids occurred—85% of progesterone and about 30% of both estrone and estradiol were found to be degraded in 24 h. The procedure developed was applied to samples from the Seine river estuary. Sex steroids were not detected in surface water; estrone was the most commonly detected steroid in sewage-treatment plant effluent, with levels ranging from 1.8 to 8.3 ng L^{-1}. Synthetic estrogens (ethynylestradiol and mestranol) and progestagens (levonorgestrel and norethindrone) were never detected, whatever the sampling season. Overall, for 162 out of 168 measurements levels were below the detection limits of the developed procedure.

Keywords Estrogens · Progestagens · Solid-phase extraction · Sewage · Surface water

Introduction

Numerous chemicals discharged into aquatic ecosystems via wastewater-treatment plants (WWTP) have been shown to mimic endogenous sex steroid hormones. Examples include phthalates, alkylphenols and their ethoxylated precursors, and hormones (both natural and synthetic). The last of these compounds, which include estrogens and progestagens, are obviously the most potent endocrine disruptors. So far, research has focused mainly on estrogenic effects, and an increasing number of papers has reported the estrogenicity of WWTP effluents. Several adverse effects, for example induction of vitellogenin [1–6] or adverse alterations of gonads [4], have been reported in male fish exposed to WWTP discharge. In-vitro tests performed on fractionated WWTP effluents showed that the natural estrogens estrone and estradiol, and the synthetic contraceptive ethynylestradiol, were the main contributors to the estrogenic potency of sewage discharge [7, 8].

The aim of this study was to develop an analytical procedure enabling determination of sex steroids in WWTP effluents and in surface water, to study their occurrence in the Seine river estuary (France). Two methodological key points, sample storage and sample preparation, have been investigated in particular depth and are discussed. The optimized procedure has then been applied within the framework of environmental monitoring campaigns undertaken in the Seine river estuary. This research is, indeed, part of the multidisciplinary "Seine Aval II" research program, which aims to assess the water quality of the Seine river estuary and to better understand the functioning of the estuary. Although the Seine river estuary has been well studied for chemical contamination, for example PAH, PCB, heavy metals [9], there was a serious lack of data concerning emerging contaminants such as hormonal steroids. Furthermore, because evidence of endocrine disruption in fish from this estuary has been reported [10] there was a serious need to acquire data on steroid concentrations in this aquatic system.

As target compounds we selected the natural estrogens estrone, estradiol, and estriol, the synthetic estrogens ethynylestradiol and mestranol, the natural progestagen progesterone, and the synthetic progestagens norethindrone and D-norgestrel. Selection of natural hormones

P. Labadie · H. Budzinski (✉)
Laboratoire de Physico-Chimie & Toxico-Chimie des systèmes naturels (LPTC), UMR 5472 CNRS, Université Bordeaux I, 351 Cours de la Libération, 33405 Talence, France
E-mail: h.budzinski@lptc.u-bordeaux1.fr

was based on their occurrence in the human body and their potency. The synthetic compounds chosen were those most commonly used in estroprogestative treatments. Steroids are mainly excreted as conjugates (glucuronides, sulfates, etc.), which are then extensively cleaved either in the sewers or during sewage treatment, probably by the β-glucuronidase secreted by the fecal bacteria *Escherichia coli* [11–13]. Therefore, this study focused on levels of the free steroids only.

Experimental

Chemicals

Both natural and synthetic steroids were purchased from Sigma–Aldrich (St Quentin Fallavier, France). The steroids selected are listed in Table 1. Deuterated steroids (testosterone-it d3, 17β-estradiol-it d4 and 17β-ethynylestradiol-it d4, purity $>99\%$), used as internal surrogates, were obtained from CDN Isotopes (Montreal, Canada). Stock solutions were prepared in MeOH at 20 μg g^{-1} and stored at 4°C. They were found to be stable over a 6-month period ($\Delta C < 5\%$).

All solvents were either HPLC or organic residue analysis grade and were obtained from Atlantic Labo (Eysines, France).

Solid-phase extraction was carried out using either Varian BondElut C18 (endcapped 500 mg) cartridges (Interchim, Montluçon, France), IST Env+ cartridges (Bios Analytique, L'Union, France), or Waters Oasis HLB cartridges (Waters, St Quentin en Yvelines, France). LC-NH2 and silica cartridges were purchased from Supelco (St Quentin Fallavier, France) and were used for clean up of the extracts.

Whatman GF-F glass fiber filters (pore size 0.7 μm) were purchased from VWR International (Strasbourg, France). MSTFA (*N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide; Acros Organics, Noisy le Grand, France) was used as the silylation agent. Deionized water was obtained with a Milli-Q system (Millipore, Molsheim, France).

Analysis and quantification of the target steroids

The steroids were quantified by GC–MS, using internal standards and after silylation of the steroids. First, the final SPE extracts were evaporated to dryness, under a N_2 stream, at 50–60°C. The silylation reagent was prepared as follows: a mixture of 250 μL MSTFA, 15 μL mercaptoethanol and 10 mg NH_4I was left for about 10 min at 65°C, to ensure total dissolution of the catalyst ammonium iodide. Thereafter, the mixture was diluted ten times with MSTFA and this solution was used as the derivatization reagent: 30 μL of the reagent was added to each extract and the samples were left at 65°C for 30–40 min. Then, 20 μL of isooctane was added and the samples were injected.

Separation and detection of the analytes were achieved using an Agilent GC system (6890 series) coupled with an Agilent MS detector (5973 series). The separation was performed on an Agilent HP5-MS column (length 30 m; internal diameter 250 μm; stationary phase thickness 0.25 μm) with the following temperature program: 90°C, 1 min; 7.5° min^{-1} to 290°C, isothermal 5 min. The injection volume was 2.5 μL (splitless; pulsed pressure 25.0 psig) and the injector temperature was 250°C. The purge flow was 60 mL min^{-1} for 1.5 min.

Deuterated steroids were used as internal surrogates: testosterone-d3 (m/z 435) for non-phenolic analytes, 17β-estradiol-d4 (m/z 420) for natural estrogens, and 17α-ethynylestradiol-d4 (m/z 429) for synthetic estrogens. For each series of analyses a response factor (relative to the appropriate surrogate) was calculated for each analyte; a mixture of standards, containing about 25 ng of each analyte and surrogate standard (exact amount checked gravimetrically), was used for this purpose. Moreover, these response factors were found to be constant (within 10%) over a wide range of injected analyte quantity (from 20 pg to 2 ng). In environmental samples, for each steroid, two ions were used for quantification (QI) and confirmation (CI) purposes (Table 2). For a given peak the ratio QI/CI was compared with that obtained with an authentic standard analyzed in full-scan mode, before definitive attribution of the peak. For each series, blank samples were run and steroid levels were systematically lower than 0.2 ng (per blank sample).

Optimization of solid-phase extraction conditions

SPE sorbent

Recoveries of the selected estrogens and progestagens were determined for three sorbents: the silica-based

Table 1 Target analytes

	Trivial name	Abbreviation	Type	CAS registry number
Estrogens	Estrone	E1	N	53-16-7
	17β-Estradiol	E2	N	50-28-2
	Estriol	E3	N	50-27-1
	17α-Ethynylestradiol	EE2	S	57-63-6
	Mestranol	MeEE2	S	72-33-3
Progestagens	Progesterone	Pg	N	57-83-0
	D-norgestrel	DN	S	797-63-7
	19-Norethindrone	No	S	68-22-4

Table 2 Ions used for the identification and quantification of the target analytes

Steroid	Internal standard	Identification and quantification ion (m/z)	Confirmation ion (m/z)
E1	E2-d4	414	399
E2	E2-d4	416	285
E3	E2-d4	504	386
EE2	EE2-d4	425	440
MeEE2	EE2-d4	367	227
Pg	T-d3	458	443
DN	T-d3	456	316
No	T-d3	442	287

Varian BondElut C18 and the polymeric sorbents Oasis HLB and IST Env+. The effect of flow rate (5–20 mL min^{-1}) and the breakthrough volume were assessed for the polymeric sorbents.

Before sample analysis BondElut C18 and Oasis HLB sorbents were conditioned with 2×4 mL methanol then 10 mL deionized water. Elution was also performed with 2×5 mL methanol. The Env+ sorbent was conditioned by passing, successively, 4 mL ethyl acetate–acetone (1:1, *v*/*v*), 4 mL methanol, and, eventually, 4 mL deionized water for equilibration. Ethyl acetate–acetone (1:1, *v*/*v*; 10 mL) was used for elution.

Sample clean up

In an attempt to improve the limits of detection of the procedure, three clean-up procedures were compared:

1. *Water/methanol mixture*: before drying, the Env+ cartridges were rinsed with 4 mL H_2O–MeOH (1:1, *v*/*v*), and the effluent discarded.
2. *Silica cartridges*: the Env+ extract was first evaporated to dryness under a nitrogen stream, at 40–50°C. The residue was then dissolved in 2 mL cyclohexane–acetone (65:35, *v*/*v*) and passed through a silica cartridge previously conditioned with cyclohexane and cyclohexane–acetone; steroids were directly collected and further eluted with 2 mL cyclohexane–acetone (method adapted from Ref. [14]).
3. *Aminopropyl cartridges*: as for silica cartridges, the Env+ extract was first evaporated to dryness. It was then dissolved in 2 mL ethyl acetate–methanol (4:1, *v*/*v*) and passed through a LC-NH_2 cartridge; steroids were directly collected and further eluted with 2 mL ethyl acetate–methanol [15].

WWTP effluent sample storage

The stability of the selected steroids in WWTP effluent during storage in bottled water was evaluated. For this purpose, a mixture of steroids was added (final concentration 100 ng L^{-1} for each analyte) to an 8-L WWTP effluent sample from the town of Tancarville (dissolved organic carbon 7.70 mg L^{-1}; particulate organic carbon: 1.13 mg L^{-1}; particulate material (>0.7 μm) 3.2 mg L^{-1}; pH 8.0, conductivity 1,152 μS cm^{-1}, salinity 0.3‰). The sample was shaken vigorously and left to equilibrate for 1 h at room temperature, because Lai et al. [16] have shown that maximum sorption occurred after 1 h. The initial concentration of each compound in the dissolved phase was then determined as described above.

Subsequently, the remaining effluent was divided into four aliquots, each one undergoing a different treatment—no preservative added, 1% formaldehyde added, 1% methanol added, and acidification to pH 3–3.5 with HCl 3 mol L^{-1}. All four bottles were stored at 4°C, in the dark. These storage methods are widely used in environmental steroid analysis [17]. After 24, 48 h and 7 days of storage, 200-mL aliquots were taken in each bottle and triplicate measurements of the steroids levels were performed using Env+ sorbent after filtration on GF/F filters. Cartridges were rinsed with 4 mL water–methanol (1:1, *v*/*v*) before drying and elution with 10 mL acetone–ethyl acetate (1:1, *v*/*v*).

Sampling and analysis of surface water and WWTP effluents

From March 2002 to November 2002, surface water and WWTP effluent samples were collected every 2 months at six stations located in the Seine river estuary: Poses (surface water (SW), kilometric point downstream of Paris (kp): 200), Caudebec (SW, kp: 313), Honfleur (SW, kp: 355), Elbeuf (WWTP, kp: 220; 110,000 population equivalents), Rouen (WWTP, kp: 245, 450,000 population equivalents), and Tancarville (WWTP, kp: 330). All three WWTP included secondary biological treatment.

Amber bottles were used and methanol or formaldehyde was immediately added to the samples (1%, *v*/*v*). The bottles were kept in the dark at 4°C until analysis, within 24 h of collection. Before the SPE step, 1-L water samples were filtered through pyrolyzed (400°C, 6 h) GF/F glass fiber filters. About 25 ng of each deuterated surrogate (T-d3, E2-d4 and EE2-d4) was added to the sample before it was passed trough the Env+ cartridges at a flow rate of 20 mL min^{-1}. Cartridges were rinsed with MeOH–H_2O and subsequent drying and elution were performed as mentioned above.

Results and discussion

SPE conditions: recovery percentages, influence of the flow rate and breakthrough volume

The recovery rates for three different sorbents are presented in Table 3. All three sorbents had relatively good extraction efficiency for the selected steroids. However, slight breakthrough was observed when E3, the most polar of the investigated steroids (K_{ow} = 2.8, [16]), was

extracted on Env+. Conversely, extraction of this compound by the Oasis HLB sorbent was satisfactory, probably because of a higher capacity factor. Similar results were obtained by Lopez de Alda and Barceló [18].

Using Env+ sorbent (200-mg cartridges), mean recoveries from spiked WWTP effluent (25 ng L^{-1} for each compound) exceeded 80% for all analytes. Results were similar to those obtained with Milli-Ω water; the only potential matrix effect observed with the effluent was, perhaps, the 1.5-fold increase of E3 recovery (90 vs. 60% for Milli-Ω). Relative standard deviations were lower than 15% for both matrices.

Recovery of steroids from 1-L samples was determined for the Env+ sorbent at two different flow rates, 5 and 15–20 mL min^{-1}. No significant differences were observed between the two experimental conditions. Therefore, subsequent extractions were carried out at 15–20 mL min^{-1}, to reduce the duration of the analytical procedure.

Recovery rates were also compared for several sample volumes, ranging from 1 L to 4 L. For the Env+ sorbent the results presented in Table 4 indicate that estrogen breakthrough occurs for sample volumes between 2 L and 4 L, irrespective of the amount of sorbent. Indeed, although good recoveries were achieved when processing a 2-L sample on a 200 mg cartridge, severe breakthrough occurred when analyzing a 4-L sample with a 500-mg cartridge—recovery of most estrogens was below 10%. MeEE2 was the only estrogen correctly retained by the Env+ sorbent, probably because of its higher hydrophobicity (log $K_{ow} = 4.7$ for MeEE2 whereas log $K_{ow} = 3.4$ for E1 [16]). Although calculated K_{ow} of the investigated progestins are in the same range as those of natural estrogens (3.0 for No, 3.4 for DN, and 3.7 for Pg, [19]) no breakthrough was observed for these steroids. This is perhaps a consequence of their non-phenolic structure, leading to different interactions with the sorbent. In contrast with the Env+ sorbent, which was not suitable for analysis of samples > 2 L, the Oasis HLB sorbent (200 mg) resulted in good extraction recoveries for all the analytes investigated, even E3. The latter sorbent can therefore be used when large volumes of water must be analyzed (i.e. surface water).

Table 3 Comparison of percentage recovery (mean value ± standard deviation, $n = 3$) obtained from analysis of Milli-Ω water samples (1 L) spiked at 50 ng L^{-1} with each analyte and extracted on three different sorbents: BondElut 200 mg, Env+ 200 mg, Oasis HLB 200 mg

Compound	BondElut C18	ENV+	Oasis HLB
E1	101 ± 10	97 ± 2	88 ± 15
E2	101 ± 1	91 ± 1	102 ± 7
E3	70 ± 5	60 ± 5	93 ± 13
EE2	85 ± 8	110 ± 10	83 ± 11
MeEE2	90 ± 5	105 ± 6	78 ± 12
Pg	96 ± 9	100 ± 8	76 ± 8
DN	97 ± 3	98 ± 5	77 ± 6
No	92 ± 2	95 ± 3	108 ± 9

Sample clean up: limits of detection

To improve limits of detection of the analytes, several procedures were tested to clean up the extracts obtained from the Env+ cartridges. The use of NH_2 and silica cartridges significantly reduced the amounts of co-extracted compounds. Rinsing the Env+ sorbent with 4 mL methanol–water (1:1, *v*/*v*) before the drying step, although slightly less effective than the previous procedures, also gave good results; the noise could be largely reduced and limits of detection were reduced by a factor 5–8, depending on the analyte. Hence, the latter method was chosen for subsequent analyses, because of both its simplicity and its rapidity.

Limits of detection for the estrogens of the developed procedure ($S/N = 3$) ranged from 0.3 to 8.0 ng L^{-1}, depending on the analyte and on the matrix. The best results were obtained for E2 in surface water, and the highest limits of detection were obtained for E3 in WWTP effluent. Limits of detection for the progestagens were higher, ranging from 1.0 to 8.0 ng L^{-1}. The limits of detection depended both on the matrix composition and derivatization efficiency, and large inter-sample variability was observed; for instance, calculated limits of detection for E3 ranged from 0.8 to 8.0 ng L^{-1}.

Sample storage

The aim of this pilot study was to provide data concerning the stability of sex steroids in WWTP effluent during short-term storage (1 week). As can be seen from the results presented in Table 5, serious problems of stability can arise during such a short period of time. When no preservative was added, severe losses of natural steroids occurred. Indeed, in such storage conditions, approximate half lives of natural steroids ranged from 8 h (Pg) to 12 days (E3), whereas intermediate half lives of, respectively, 87 h (2.9 days) and 69 h (3.6 days) were obtained for E1 and E2; it should be emphasized that these values are merely approximate estimates, because only four points were used for the calculations. No clear oxidation of E2 to E1 was observed, because apparent degradation rates were similar for E1 and E2 (both of which were present at t_0). Synthetic estrogens and progestagens were found to be stable over the whole duration of storage. The rapid loss of Pg was not because of sorption on to particulate matter. Indeed, this was checked by determining steroid levels in the suspended solid phase. Briefly, microwave-assisted extraction was performed using MeOH–H_2O (55:45, *v*/*v*) as extraction solvent; the so-obtained extracts were then purified on Env+ SPE cartridges, as previously described for water samples. Whatever the assay (no preservative, MeOH, HCl, or formaldehyde), no analyte could not be detected in the particulate fraction (> 0.7 μm), suggesting a negligible role of adsorption in the removal of natural steroids from the dissolved phase. This was, perhaps, because of the small amount of sus-

Table 4 Comparison of percentage recovery (mean value ± standard deviation, $n = 3$) obtained from analysis of Milli-Ω water samples of different volumes (2–4 L) spiked at 50 ng L^{-1} with each analyte and extracted on Env+ and Oasis HLB sorbents

Sample volume	2 L	4 L	4 L	4 L
Sorbent	Env+ 200 mg	Env+ 200 mg	Env+ 500 mg	Oasis HLB 200 mg
E1	97 ± 3	3 ± 2	4 ± 1	75 ± 7
E2	99 ± 3	1 ± 0	10 ± 2	84 ± 3
E3	47 ± 2	0 ± 0	0 ± 0	80 ± 4
EE2	110 ± 2	6 ± 1	8 ± 2	71 ± 14
MeEE2	109 ± 11	85 ± 1	85 ± 10	73 ± 19
Pg	74 ± 2	75 ± 2	96 ± 4	93 ± 5
DN	115 ± 15	97 ± 10	108 ± 8	80 ± 5
No	74 ± 2	98 ± 10	107 ± 5	109 ± 5

pended matter (3.5 mg L^{-1}); previous studies dealing with binding of waterborne steroids to solid phase involved particle loads one order of magnitude higher [16], which may have favored sorption.

Addition of 1% MeOH (*v*/*v*) was not able to effectively slow down biotic degradation of natural steroids during storage for 7 days. Conversely, formaldehyde was found to block bacterial activity in the WWTP effluent, because no significant loss of steroid was observed; similar results were published by Baronti et al. [20] for longer storage times. Acidification to pH 3.5 also blocked biologically mediated degradation of estrogens and progestagens, but coeluted matrix interferences strongly disrupted the quantification, as can be seen from high C/Co values (greater than 100%) and from standard deviations (Table 5) higher than those observed with the other preservative treatments. As a consequence, addition of formaldehyde was used for long-term storage (> 24 h), but MeOH was also used when analysis was performed within 24 h and when estrogens were the only analytes of interest.

Occurrence of sex hormones in the Seine river estuary

In most samples measured in this study levels of estrogens and progestagens were below the limits of detection (Table 6). Sex steroids were not detected in any surface water sample, irrespective of salinity, sampling season, or flow rate of the river. In an attempt to improve the limits of detection, an extraction procedure for 4-L samples was developed, as described above, but the limits of detection were not improved because the baseline noise was higher than with the 1-L samples.

Sex steroids were not systematically detected in the three WWTP effluents investigated; similar observations have been reported after several other studies [12, 20–23]. The sex steroid most commonly detected was E1 (1.8–8.3 ng L^{-1}) (Fig. 1). The slight seasonal variability of E1 levels in WWTP effluent may, perhaps, be partially explained by differences of efficiency in wastewater treatment—the lowest value was measured in July, warmest month of this study. Another, more likely, explanation is that this variability is because our study is based on discrete sampling and WWTP effluent composition is highly variable [3, 24]. E2 was never detected, probably because of its oxidation to E1 during sewage treatment and E3 was detected only once, at a low concentration (3.5 ng L^{-1}).

Synthetic steroids were not detected in the WWTP effluents investigated, perhaps as a consequence of the higher detection limits obtained for EE2, MeEE2, DN, and No. This may also be because of the relatively small amounts consumed for contraception purposes (approx. 30 μg EE2 and 150 μg DN or No per woman and per day). For comparison, daily production of natural estrogens is close to 150 μg/woman/day [11] and that of progesterone ranges from 3 to 30 mg/woman/day [11].

As far as we are aware, the fate of Pg within WWTP has never been reported. In this study, we observed rapid degradation of Pg in spiked WWTP effluent samples. One can therefore assume that this steroid is rapidly eliminated in sewers or during sewage treatment and, hence, Pg levels in WWTP effluents are probably well

Table 5 Effects of various preservation treatments (applied to the raw water) on the stability of sex steroids in the dissolved phase of WWTP effluent (results are expressed as mean percentage of the initial concentration ± standard deviation, $n = 3$)

Assay	No preservative			MeOH			HCl			Formaldehyde		
Time	24 h	48 h	7 days	24 h	48 h	7 days	24 h	48 h	7 days	24 h	48 h	7 days
E1	70 ± 5	67 ± 1	27 ± 1	79 ± 14	77 ± 6	37 ± 2	98 ± 8	106 ± 4	189 ± 17	92 ± 3	96 ± 9	113 ± 5
E2	79 ± 6	72 ± 6	18 ± 3	90 ± 8	77 ± 4	31 ± 1	90 ± 8	127 ± 7	106 ± 14	100 ± 7	94 ± 7	109 ± 3
E3	105 ± 14	112 ± 3	62 ± 1	111 ± 7	115 ± 10	65 ± 3	153 ± 19	115 ± 13	174 ± 24	109 ± 1	98 ± 7	107 ± 3
EE2	99 ± 15	101 ± 6	104 ± 8	99 ± 14	103 ± 4	92 ± 1	92 ± 5	94 ± 2	86 ± 9	92 ± 1	81 ± 5	96 ± 3
MeEE2	98 ± 12	102 ± 20	74 ± 20	109 ± 4	103 ± 10	100 ± 14	107 ± 11	113 ± 20	105 ± 11	98 ± 11	84 ± 8	94 ± 4
Pg	14 ± 2	8 ± 4	2 ± 1	21 ± 5	18 ± 8	1 ± 1	73 ± 7	61 ± 6	87 ± 19	73 ± 2	67 ± 3	82 ± 5
DN	97 ± 17	122 ± 1	81 ± 5	106 ± 18	94 ± 7	88 ± 2	81 ± 4	94 ± 7	85 ± 38	86 ± 6	94 ± 7	107 ± 10
No	110 ± 2	120 ± 10	74 ± 1	127 ± 28	128 ± 9	92 ± 2	116 ± 25	128 ± 16	160 ± 7	100 ± 6	104 ± 15	120 ± 5

Table 6 Occurrence of selected sex steroids in surface water and WWTP effluent in the Seine river estuary

Sampling location	Kilometric point	Sampling month	Concentration (ng L^{-1})							
			E1	E2	E3	EE2	MeEE2	Pg	DN	No
Poses	200	March	< 1.3	< 1.0	< 1.9	< 1.8	< 1.6	< 2.5	< 4.0	< 2.5
		May	< 1.2	< 0.5	< 2.0	< 1.1	< 1.0	< 2.7	< 5.9	< 2.5
Caudebec	313	March	< 1.3	< 1.5	< 1.9	< 1.8	< 1.6	< 2.5	< 4.0	< 2.5
		May	< 0.8	< 0.5	< 2.2	< 1.0	< 0.4	< 2.7	< 3.8	< 1.3
Honfleur	355	March	< 1.2	< 1.0	< 1.0	< 1.5	< 1.0	< 2.2	< 4.5	< 1.5
		May	< 1.1	< 0.8	< 1.2	< 1.3	< 0.8	< 2.0	< 5.0	< 1.3
Elbeuf (WWTP)	220	March	< 2.0	< 1.9	< 4.5	< 3.0	< 3.5	< 5.0	< 4.5	< 5.0
		May	4.3 ± 0.9	< 3.8	< 8.0	< 5.3	< 5.4	< 8.0	< 7.2	< 6.5
		July	< 3.5	< 0.6	< 4.9	< 0.8	< 0.7	< 4.5	< 6.7	< 3.2
		September	< 0.5	< 0.4	< 0.8	< 0.8	< 0.9	< 3.5	< 6.1	< 4.3
		November	< 4.3	< 2.4	< 5.6	< 1.1	< 1.1	< 4.3	< 6.3	< 1.9
Rouen (WWTP)	245	March	< 1.8	< 1.9	< 4.0	< 2.9	< 3.0	< 3.5	< 3.8	< 5.2
		May	< 3.0	< 3.8	< 8.0	< 5.3	< 5.0	< 7.0	< 7.2	< 5.0
		July	< 3.3	< 0.5	3.5 ± 1.0	< 1.1	< 1.1	< 4.3	< 6.0	< 3.1
		September	< 0.5	< 0.4	< 2.1	< 1.0	< 0.7	< 3.5	< 5.8	< 4.0
		November	< 3.4	< 2.5	< 7.3	< 1.2	< 0.9	< 6.8	< 2.5	< 2.5
Tancarville (WWTP)	330	March	< 2.8	< 2.5	< 3.0	< 2.5	< 2.7	< 4.0	< 3.2	< 4.5
		May	4.2 ± 1.0*	< 0.8	< 1.8	< 0.7	< 1.2	< 1.7	< 3.2	< 1.1
		July	1.8 ± 0.3	< 0.3	< 3.6	< 1.0	< 0.6	< 3.7	< 5.0	< 3.3
		September	8.3 ± 1.0*	< 0.3	< 1.9	< 0.7	< 0.7	< 3.7	< 4.3	< 3.9
		November	4.9 ± 0.2	< 1.4	< 5.0	< 1.0	< 1.1	< 5.0	< 5.0	< 1.5

*means > limit of quantification.

below the limits of detection of the analytical method presented in this paper (Table 6). Vanderford et al. [25] reported concentrations of Pg in surface waters impacted by municipal effluent ranging from 14 to 44 ng L^{-1}, but these results were viewed with caution because of high concentrations in their blank sample (average level 217 ng L^{-1}).

Conclusion

An analytical procedure has been developed that enables determination of five estrogens and three progestagens in waste water and surface water with good recoveries and limits of detection ranging from 0.3 to 8.0 ng L^{-1}, depending on the matrix and on the analyte. It was found that sample storage was a critical step in steroid analysis in water samples; formaldehyde (1%, *v*/*v*) must be added to the samples immediately after collection if natural steroids are to be analyzed. Otherwise, dramatic degradation of Pg and, to a lesser extent, E1 and E2 occurs. Application of the developed procedure to the Seine river estuary revealed no occurrence of steroids in surface water, the levels being lower than the limits of detection. The natural estrogens E1 and E2 were detected, at concentrations below 10 ng L^{-1}, in sewage-treatment plant effluents only.

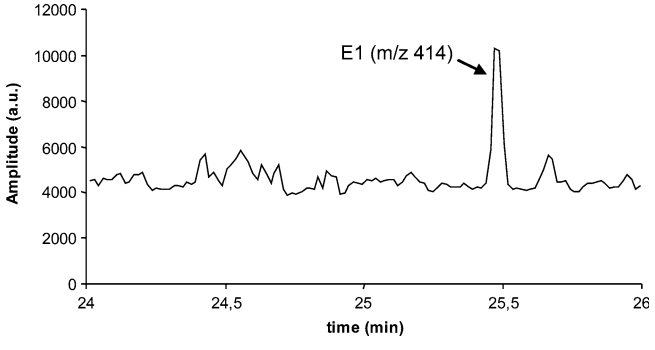

Fig. 1 GC–MS chromatogram resulting from analysis of estrone ($m/z = 414$) in treated sewage (Tancarville, September 2002)

Acknowledgements The authors would like to thank the "Seine Aval" research program for financial support. Pierre LABADIE was funded by the CNRS and the Aquitaine Région.

References

1. Routledge EJ, Sheahan D, Desbrow C, Brighty GC, Waldock MJ, Sumpter JP (1998) Environ Sci Technol 32:1559–1565
2. Tilton F, Benson WH, Schlenck D (2002) Aquat Toxicol 61:211–225
3. Rodgers-Gray T, Jobling S, Morris S, Kelly C, Kirby S, Janbakhsh A, Harries JE, Waldock MJ, Sumpter JP, Tyler CR (2001) Environ Sci Technol 34:1521–1528
4. Rodgers-Gray T, Jobling S, Kelly C, Morris S, Brighty G, Waldock MJ, Sumpter JP, Tyler CR (2001) Environ Sci Technol 35:462–470
5. Larsson DGJ, Adolfsson-Erici M, Parkkonen J, Pettersson M, Berg AH, Olsson PE, Förlin L (1999) Aquat Toxicol 45:91–97
6. Solé M, Lopez de Alda MJ, Castillo M, Porte C, Ladegaard-Pedersen K, Barcelo D (2000) Environ Sci Technol 34:5076–5083
7. Desbrow C, Routledge EJ, Brighty G, Sumpter JP, Waldock MJ (1998) Environ Sci Technol 32:1549
8. Aerni HR, Kobler B, Rutishauser BV, Wettstein FE, Fischer R, Giger W, Hungerbühler A, Marazuela MD, Peter A, Schönenberger R, Vögeli AC, Suter MJF, Eggen RIL (2003) Anal Bioanal Chem 378:688–696

9. Tronczynski J, Munschy C, Moisan K (1999) Seine Aval programme, vol 12. Editions Ifremer, Plouzané, France
10. Minier C, Levy F, Rabel D, Bocquené G, Godefroy D, Burgeot T, Leboulanger F (2000) Mar Environ Res 50:373–377
11. D'Ascenzo G, Di Corcia A, Gentili A, Mancini R, Mastropasqua R, Nazzari M, Samperi R (2003) Sci Total Environ 302:199–209
12. Belfroid AC, Van der Horst A, Vethaak AD, Schäfer AJ, Rijs GBJ, Wegener J, Cifino WP (1999) Sci Total Environ 225:101–108
13. Ternes TA, Kreckel P, Mueller J (1999) Sci Total Environ 225:91–99
14. Ternes TA, Stumpf M, Mueller J, Haberer K, Wilken RD, Servos M (1999) Sci Total Environ 225:81–90
15. Le Bizec B, Marchand P, André F (2000) Ann Toxicol Anal 12:56–63
16. Lai KM, Johnson KL, Scrimshaw MD, Lester JN (2000) Environ Sci Technol 34:3890–3894
17. Lopez de Alda M, Barceló D (2001) Fresenius J Anal Chem 371:437–450
18. Lopez de Alda M, Barceló DJ (2001) J Chromatogr A 938:145–153
19. KowWin program (1999) Environmental Science Centre, Syracuse Research Corporation, New York, USA (http://www.syrres.com/esc/kowwin.htm) (used in May 2004)
20. Baronti C, Curini R, D'Ascenzo G, Di Corcia A, Gentili A, Samperi R (2000) Environ Sci Technol 34:5059–5066
21. Kuch HM, Ballschmiter K (2000) Fresenius J Anal Chem 366:392–395
22. Huang CH, Sedlak DL (2001) Environ Toxicol Chem 20:133–139
23. Snyder SA, Keith TL, Verbrugge DA, Snyder EM, Gross TS, Kannan K, Giesy JP (1999) Environ Sci Technol 33:2814–2820
24. Williams RJ, Johnson AC, Smith JJL, Kanda R (2003) Environ Sci Technol 37:1744–1750
25. Vanderford BJ, Pearson RA, Rexing DJ, Snyder SA (2003) Anal Chem 75:6265–6235