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Journal of Chromatography A, 1024 (2004) 177–185
JOURNAL OF CHROMATOGRAPHY A
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Determination of natural and synthetic estrogens in water by gas chromatography with mass spectrometric detection☆

J.B. Quintana, J. Carpinteiro, I. Rodríguez, R.A. Lorenzo, A.M. Carro, R. Cela*

Departamento de Química Analítica, Nutrición y Bromatología, Universidad de Santiago de Compostela, Santiago de Compostela 15708, Spain

Received 7 March 2003; received in revised form 7 October 2003; accepted 20 October 2003

Abstract

A procedure for the determination of six natural and synthetic estrogens (diethylstilbestrol, estrone, 17β-estradiol, mestranol, 17α-ethinylestradiol and estriol) in water samples is described. Samples, up to 2000 ml, were concentrated using Oasis HLB solid-phase extraction cartridges. Analytes were derivatized with *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide and determined by GC–MS or GC–MS–MS. The reactivity of several silylation reagents versus aliphatic and aromatic hydroxyl groups contained in the structure of the selected analytes was evaluated. Influence of parameters such as sample pH, nature of the water samples and derivatization conditions on the performance of the whole analytical procedure was systematically studied. Under optimal conditions, quantification limits between 1 and 3 ng/l were achieved for the determination of the considered estrogens in sewage water.
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Keywords: Water analysis; Derivatization, GC; Environmental analysis; Estrogens; Steroids

1. Introduction

Urinary excretion of natural estrogens (e.g. estrone, 17β-estradiol and estriol) and synthetic compounds used in medicine, as contraceptives and in some hormonal therapies (e.g. 17α-ethinylestradiol, mestranol), or in veterinary, as growth promoters of farm animals (e.g. diethylstilbestrol), together with their incomplete removal in sewage treatment plants, have caused the presence of several estrogens and related compounds in the aquatic environment at the ng and sub-ng per litre level [1–3]. Even at such low concentrations, some of these compounds present activity as endocrine disrupters being responsible for the feminisation of certain aquatic organisms [4].

Assessment of the environmental impact of estrogenic compounds and improvement of water treatment processes require analytical methods which allows the reliable determination of these species at the ng/l level. In most cases, these methods consist of an extraction and pre-concentration step followed by the determination of the analytes using gas (after derivatization of the native species) or liquid chromatography coupled with mass spectrometry [5]. Normally, the lower detection limits are achieved using an off-line extraction step and tandem MS–MS detection. Furthermore, depending on the selectivity of the extraction step and the level of interferences presented in the sample, a clean-up of the organic step is advisable before its injection in the chromatographic system. For each one of these steps: extraction, derivatization and clean-up different sorbents and reagents have been proposed in the literature.

Regarding the concentration of water samples, off-line solid-phase extraction (SPE) is one of the most used approaches. In this case higher recoveries and larger breakthrough volumes have been reported with C_{18} type materials than with Amberlite and other commercial styrene-divinylbenzene polymers [6–8]; however, when medium sample volumes are considered (e.g. 500 ml), quantitative recoveries for diethylstilbestrol were not achieved with any of these sorbents [7]. Conversely to these results, using on-line combinations of SPE with HPLC, better recoveries have been achieved using some polymeric materials such as HySphere and PLRP-S than with C_{18} materials, [9,10]. A

☆ Presented at the Second Meeting of the Spanish Society of Chromatography and Related Techniques, Barcelona, 26–29 November 2002.
* Corresponding author. Tel.: +34-981-563100x14271; fax: +34-981-547141.
E-mail address: qnrctd@usc.es (R. Cela).

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doi:10.1016/j.chroma.2003.10.074

third group of authors has proposed the use of home filled cartridges containing Lichrolut EN and C_{18} [6,11]. In this case, estrone, 17β-estradiol and 17α-ethinylestradiol were quantitatively recovered from 1 l volume samples; however, this mixed sorbent failed in the retention of the polar estrogen 16α-hydroxyestrone [11]. Retention of estriol and diethylstilbestrol in this mixed sorbent has not been studied.

In spite of the weak acidity of estrogenic compounds, it has been demonstrated that recoveries of solid-phase extraction, using C_{18} sorbents, decreased when the pH of water samples increased from 2 to 6 [8]. The problem of performing the SPE at acid pH is that humic acids are strongly retained on reverse phase sorbents; as a consequence yellowish extracts, containing a high level of interferences, are obtained [12,13].

When GC is used as separation technique, estrogens need to be derivatized previously to their injection in the chromatographic system. Although some authors have reported the direct derivatization of the analytes in the aqueous samples, e.g. using acetic anhydride [14], in most cases the native species are derivatized after their extraction from the matrix. Several silylation agents such as *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide (MTBSTFA) [8,15], bis(trimethylsilyl) trifluoroacetamide (BSTFA) [16], and *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA) [6,11,17], alone or in combination with a small proportion of different catalyzers such as trimethylsilylimidazole (TMSI) and trimethylchlorosilane (TMCS), have been employed for the derivatization of the hydroxyl groups contained in the estrogens moiety. Differences among these reagents are based: first, on their reactivity towards aromatic and aliphatic hydroxyl groups and second, in the stability of the obtained derivatives. However, a systematic optimization of the derivatization conditions, considering the effects of time, temperature, matrix and volume of derivatization reagent was normally not performed.

In this work, an off-line solid-phase extraction procedure, using a modified polymeric sorbent, in combination with GC–MS and GC–MS–MS detection was proposed for the analysis of estrogens in waters samples. All steps comprised in the analytical procedure were systematically evaluated. In first term, the reactivity of three silylation reagents towards the hydroxyl groups of six natural and synthetic estrogens (diethylstilbestrol, estrone, 17β-estradiol, 17α-ethinylestradiol, mestranol and estriol) was compared and once the derivatization reagent was chosen, the influence of different parameters in the yield of the process was systematically investigated. Breakthrough volumes and effect of sample pH in the recoveries of the solid-phase extraction step and in the cleanliness of the obtained extracts were presented; furthermore, the need or not of a clean-up step, was discussed. Finally, the developed method was applied to the determination of estrogens in influent and effluent sewage water samples.

2. Experimental

2.1. Reagents and standards

HPLC-grade methanol and ethyl acetate were supplied by Merck (Darmstadt, Germany). Standards of diethylstilbestrol, estrone, 17β-estradiol, 17α-ethinylestradiol, mestranol and estriol were obtained from Aldrich (Milwaukee, WI, USA). [$^{2}H_4$]17β-estradiol (17β-estradiol-d_4) (deuterium was introduced in positions 2, 4 and 16) was obtained from Cambridge Isotope Laboratories (Andover, MA, USA). MTBSTFA, BSTFA, BSTFA containing 1% of TMCS, MSTFA and TMSI, were obtained from Supelco (Bellefonte, PA, USA). Individual stock solutions of the estrogenic compounds were prepared in methanol. Diluted standards and mixtures of the investigated compounds were prepared in both methanol (when used to spike water samples) and ethyl acetate (when considered as calibration solutions after their silylation with the considered reagent). SPE cartridges containing 60 mg of Oasis HLB and 500 mg cartridges containing different normal phase sorbents (silica, alumina, florisil, cianopropyl and aminopropyl) were obtained from Waters (Milford, MA, USA) and used as received.

2.2. Samples and sample concentration

Spiked and non-spiked Milli-Q, river and sewage influent and effluent water samples were used in this study. Sewage samples were taken in the influent and effluent of a treatment plant equipped with primary and biological treatments. River and sewage samples were filtered after received using 0.45 μm pore size cellulose filters. After filtration, pH was measured and depending on the experiment adjusted at the selected value using 0.1 M solutions of HCl or NaOH. Then the internal standard, 17β-estradiol-d_4, was added to the samples at the 75 ng/l level. Furthermore, in recovery experiments samples were spiked with the six estrogens considered in this study.

Spiked and non-spiked samples were forced to pass through the Oasis cartridge (approximately at 15–20 ml/min) that had been sequentially pre-conditioned with ethyl acetate, methanol and Milli-Q water adjusted at the same pH that the sample (3 ml each). After finishing the concentration step, cartridges were dried with a stream of nitrogen for 30 min and eluted with 3 ml of ethyl acetate. In the case of Milli-Q and river water samples this extract was reduced to 0.1 ml and submitted directly to the derivatization procedure. For sewage water samples (effluent and specially influent samples) a dark extract was obtained after the SPE step; therefore, the final volume was reduced to approximately 0.3 ml and further cleaned-up by passing it through a 500 mg silica cartridge (previously conditioned with 5 ml of ethyl acetate). Analytes were then eluted with 10 ml of ethyl acetate, whereas polar interferences remained retained on the silica sorbent. This extract was again reduced to 0.1 ml and derivatized.

Table 1
Retention times, MS and MS–MS detection parameters for the silylated estrogens

Compound	Retention time (min)		MS detection, quantification ion (m/z)[a]	MS–MS detection		
	BP-5 column	BP-1 column		Quantification ion (m/z)	Other product ion (m/z)	CID Ampere (V)
Diethylstilbestrol	16.2, 18.9	11.1, 12.1	412	383	397	0.82
Estrone	31.2	15.1	342	257	244, 314	0.49
17β-Estradiol-d_4	34.1	17.1	420	330 + 287	301	0.60
17β-Estradiol	34.2	17.1	416	326 + 285	298	0.56
Mestranol	37.9	17.8	367	193	223, 349, 352	0.44
17α-Ethinylestradiol	41.0	20.4	425	193	331, 407	0.48
Estriol	44.6	25.1	414	324	295, 311,386	0.61

[a] Also used as parent ions in MS–MS detection.

2.3. Derivatization

Calibration standards, containing increasing amounts of the analytes and a fixed concentration of the internal standard, and extracts from water samples were derivatized in a 1.5 ml GC vial. In the optimal conditions 100 μl of the estrogens in ethyl acetate were mixed with 200 μl of MSTFA. Vials were closed and placed in an oven at 85 °C for 100 min. After that, they were cooled to room temperature and injected in the chromatographic system.

2.4. Equipment

Derivatized estrogens were determined using GC–MS and GC–MS–MS. A Varian Star 3400 CX gas chromatograph (Walnut Creek, CA, USA) equipped with a BP-5 type capillary column (30 m × 0.25 mm i.d., d_f: 0.25 μm) and connected to an ion-trap mass spectrometer (Varian Saturn 4) was used in the MS detection mode. GC–MS–MS analysis were carried out using a Varian CP 3800 gas chromatograph equipped with a BP-1 type capillary column (30 m×0.32 mm i.d., d_f: 0.17 μm) connected to ion-trap mass spectrometer (Varian Saturn 2000) with capacity to perform MS–MS analysis. Injections (1–2 μl) were performed in the splitless mode with a purge time of 1 min.

In both columns the silylated compounds were separated using the following oven program: 1 min at 50 °C, first ramp at 20 °C/min to 220 °C (held for 27 min, 17 min for the BP-1 type column), second ramp at 20 °C/min to 250 °C (held for 20 min). The GC–MS interface and the ion trap temperature were set at 250 and 200 °C, respectively. Mass spectra were obtained, in the m/z interval from 100 to 550, using electron impact ionization (70 eV). Retention times and *m/z* ratios used for quantitative purposes, in MS and MS–MS detection modes, were those given in Table 1.

2.5. Quantification

Levels of estrogen compounds in spiked and non-spiked samples were determined using 17β-estradiol-d_4 as internal standard throughout the whole analytical procedure. Calibration curves were built by plotting the ratio: analyte peak area/17β-estradiol-d_4 peak area versus the analyte concentration.

3. Results and discussion

3.1. Optimization of the derivatization conditions

3.1.1. Choice of the derivatization reagent

Reactivity of different silylation reagents versus the aromatic and aliphatic hydroxyl groups contained in the structure of the estrogenic species was investigated by adding a fixed amount (100 μl) of the considered reagent (alone or in combination with a catalyzer) to a standard of the analytes in 100 μl of ethyl acetate. The mixture was heated at 60 °C for 1 h and injected in the GC–MS system in order to evaluate the structure of the obtained derivatives. Results are summarized in Table 2. The MTBSTFA reagent was able to react only the hydroxyl groups in the position 3 of the aromatic ring of all considered analytes, forming the

Table 2
Reactivity of different silylation reagents versus the hydroxyl groups of the estrogen compounds

Reagent	Silylated –OH groups					
	Diethylstilbestrol	Estrone	17β-Estradiol	Mestranol	17α-Ethinlyestradiol	Estriol
MTBSTFA	All	Aromatic	Aromatic	None	Aromatic	Aromatic
BSTFA	All	All	All	None	Aromatic	All
BSTFA (1% TMCS)	All	All	All	None	Aromatic	All
MSTFA	All	All	All	All	All	All

tert-butyldimethylsilyl derivatives. The BSTFA reagent was able to react with the aromatic hydroxyl groups of all compounds and the aliphatic hydroxyl groups of 17β-estradiol and both aliphatic –OH groups, bounded to carbons in position 16 and 17 of estriol; however, the aliphatic hydroxyl groups of mestranol and ethinylestradiol remained underivatized. Similar results were obtained using a mixture of BSTFA and TMCS (1%). Silylation of all hydroxyl groups contained in the considered analytes was achieved using MSTFA, without the addition of any catalyser, probably because of its smaller size, it can approach effectively to the hindered –OH hydroxyl groups of mestranol and ethinylestradiol which did not react with BSTFA. Independently of the employed derivatization reagent (MTBSTFA, BSTFA or MSTFA), two peaks, with the same mass spectra, were always obtained for diethylstilbestrol; it was assumed that they corresponded to the *cis* and *trans* isomers [19]. In further experiments the sum of peak areas of both peaks was used for quantitative purposes.

3.1.2. Derivatization conditions

Influence of experimental conditions: time, temperature, volume of MSTFA and proportion of catalyser (TMSI, added to the silylation reagent), in the derivatization of the estrogens was studied using an experimental design. Experimental domain points for the four factors were selected according to conditions available in the literature [2,6,8]. A hybrid design which considers the four selected variables at different levels and uses a second-order model to adjust the response surface was chosen. These kind of designs, initially proposed by Roquemore [20] and further improved by other authors [21], are the most economical in terms of the number of experiments and permit to find optimal experimental conditions using response surface methodology. In this case, the experimental matrix (Table 3) consider 3 factors on 5 levels and 1 factor on 4 levels in 16 experiences for 6 responses (peak areas for estrogen compounds).

Table 3
Design matrix used in the optimization of derivatization conditions

Experiment number	Volume, MSTFA (μl)	TMSI (%)	Time (min)	Temperature (°C)
1	133	0.6	75	95
2	58	0.2	45	71
3	58	0.2	105	71
4	210	0.2	45	71
5	210	0.2	105	71
6	58	1.0	45	71
7	58	1.0	105	71
8	210	1.0	45	71
9	210	1.0	105	71
10	133	0.6	30	40
11	133	0.6	120	40
12	20	0.6	75	40
13	245	0.6	75	40
14	133	0.0	75	40
15	133	1.2	75	40
16	133	0.6	75	60

Experiments were performed with extracts obtained for a sample (5 l) of sewage water (effluent) spiked with the studied compounds at the 2 ng/ml level. This sample was divided in fractions of 1 l. Each fraction was concentrated using a SPE cartridge, analytes were eluted with 3 ml of ethyl acetate, concentrated to 0.5 ml and the organic extracts obtained from several samples mixed. Then, 100 μl of this combined extract were placed in a vial and spiked with the corresponding volume of MSTFA (containing the appropriate proportion of TMSI). Vials were capped and placed in an oven according to conditions (time and temperature) given in Table 3. Finally they were opened and made up to 400 μl with ethyl acetate. Data (peak areas) for each compound were statistically evaluated with the software package NEMROD for Windows 95 (LPRAI, University Aix-Marseille III, France).

The most important variable was the volume of derivatization reagent; however, at the 95% confidence level, none of the four variables was statistically significant. In order to define the best range for each response, within the explorer experiment design, a multicriteria desirability function was used [21]. For the desirability of a function requiring maximization (like estrogens study), it is assumed that there is a target value for the response above which results are totally satisfied. It also exists a lower threshold below which results are not acceptable. The simultaneous optimization of the derivatization step for all compounds led to the following conditions: 100 min at 85 °C using 200 μl of MSTFA containing a 0.8% of TMSI. Due to the tedious process of combining the derivatization reagent with a small percentage of catalyser, some additional experiments were performed to prove if the presence of catalyser was really significant. Fig. 1 compares peak areas corresponding to derivatized estrogen compounds in the combined extracts of a spiked sewage water sample (effluent). Derivatization conditions were those given above, but in one set of experiments ($n = 4$ replicates) the catalyser was not added. As significant differences in the obtained peak areas were not noticed, it was decided to remove the catalyser from the derivatization scheme.

3.1.3. Stability of the silylated compounds

Normally, standards and organic extracts from water samples were derivatized immediately before their chromatographic analysis and their temporal stability was not systematically studied. However, apparent degradation of the silylated estrogens was not detected after 2 months of storage at −20 °C.

3.2. Optimization of the solid-phase extraction

The first parameter considered in the optimization of the solid-phase extraction was the volume of ethyl acetate necessary to desorb the estrogenic compounds from the SPE

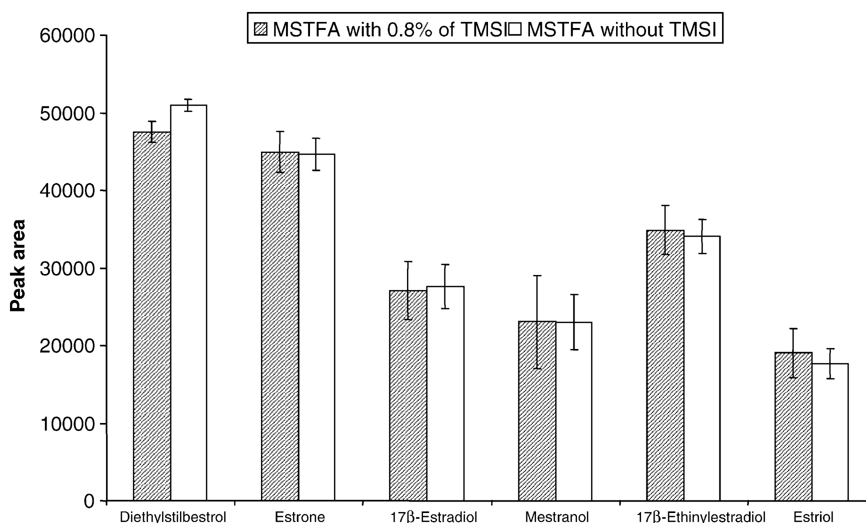

Fig. 1. Effect of TMSI in the obtained peak areas for estrogens in the extract of a spiked sewage water. Derivatization conditions: 100 μl of extract, 200 μl of MSTFA, 85 °C and 100 min.

sorbent. This solvent was chosen because of its compatibility with silylation reactions and because of its strength to elute the estrogens from reverse phase sorbents [8]. It was found that analytes were completely desorbed from the SPE cartridges with 3 ml of ethyl acetate (results not shown). Breakthrough volumes of the Oasis sorbent were investigated using spiked water samples (ca. 5 ng/ml for each analyte), containing 1% of methanol. It has been stated in the literature that due to the hydrophobic behaviour of estrogens they are prone to be adsorbed on filters and plastic materials such as the polypropylene body of the SPE cartridges given as a result non quantitative recoveries [5]. Therefore, methanol was added in order to improve solubility of the analytes, minimizing this problem, which may be specially critical at the low concentration levels that these compounds occur in the samples. Water samples were forced to pass through two cartridges sequentially connected. After finishing the enrichment step they were disconnected and processed separately. Analytes were not detected in the organic extract of the second cartridge even after the concentration of 2000 ml of Milli-Q water. The same experiment was repeated using spiked river water (2000 ml) and the effluent of the sewage treatment plant with similar results. However, in the last case cartridges were blocked after the concentration of sample volumes higher than 1500–1600 ml; therefore in case of sewage water it was decided to limit the sample volume to 1000 ml.

Recoveries of the analytical procedure were first estimated using 2000 ml of Milli-Q water with a spike of the selected compounds at 75 ng/l. Measurements were done using MS detection. As shown in Table 4, quantitative recoveries and relatively small standard deviations, specially for 17β-estradiol, were obtained for all compounds using spiked Milli-Q water samples at pH 6. Recoveries obtained for samples (spiked Milli-Q water) adjusted at pH 2 and 8 were equivalent to those obtained at pH 6 (data not shown). This retention behavior, in good agreement with the very weak acidic character of estrogens (pK_a values of 9.3 for diethylstilbestrol, between 10.3 and 10.4 for estrone, 17β-estradiol, estriol and 17α-ethinylestradiol, and 13.1 for mestranol), means that real samples can be concentrated without adjusting the pH at acidic values, as it was

Table 4
Recoveries of estrogenic species spiked over different water samples (pH = 6, spiked level 75 ng/l, $n = 4$ replicates)

Compound	Recovery (%) ± R.S.D.				
	Milli-Q water (2000 ml), without clean-up[a]	Milli-Q water (2000 ml), with clean-up[a]	River water (2000 ml), without clean-up[a]	Effluent (1000 ml), with clean-up[a]	Influent (1000 ml), with clean-up[b]
Diethylstilbestrol	97 ± 9	101 ± 9	101 ± 9	99 ± 5	88 ± 10
Estrone	104 ± 3	108 ± 4	104 ± 3	102 ± 5	83 ± 14
17β-Estradiol	98.4 ± 0.3	101 ± 2	96 ± 3	97 ± 4	94 ± 4
Mestranol	89 ± 2	102 ± 4	82 ± 3	92 ± 4	99 ± 11
17α-Ethinylestradiol	90 ± 6	97 ± 2	87 ± 1	95 ± 3	98 ± 7
Estriol	96 ± 4	87 ± 6	97 ± 7	92 ± 4	79 ± 9

[a] MS detection.
[b] MS–MS detection.

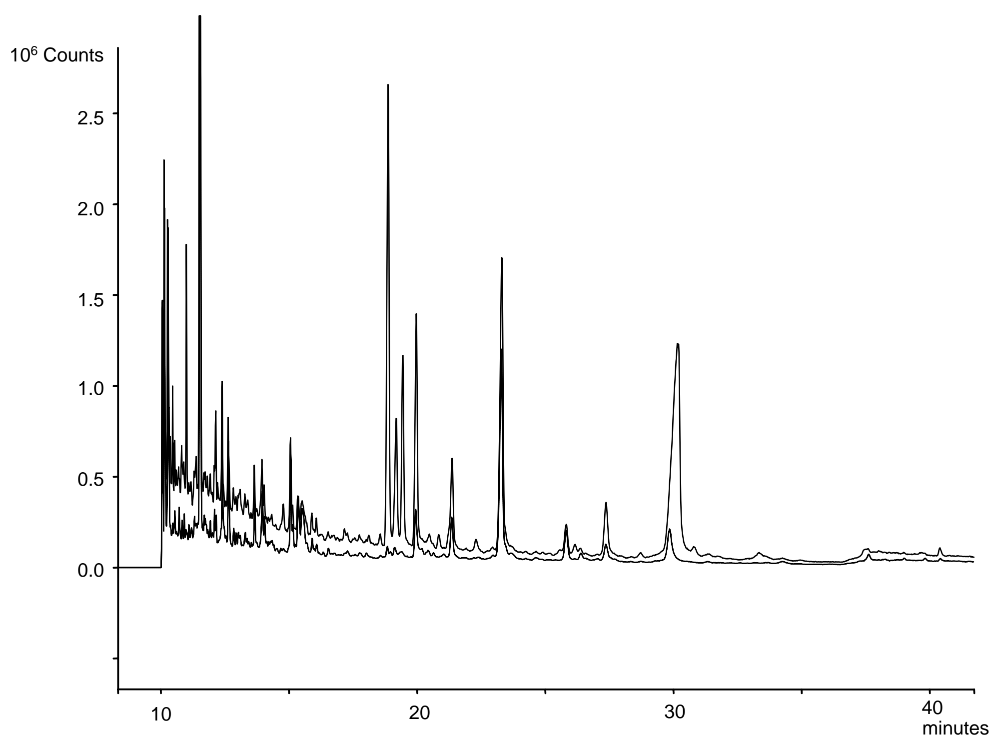

Fig. 2. TIC, GC–MS chromatogram for a sample of 250 ml of sewage water (effluent) concentrated at different pH and not submitted to the clean-up step. Solid line (sample adjusted at pH 2), dotted line (sample adjusted at pH 6).

recommended for the concentration of 17α-ethinylestradiol using C_{18} and polymeric sorbents [8]. Performing the extraction at neutral pH values has the advantage of reducing the retention of humic acids on the SPE cartridge leading to cleaner extracts, Fig. 2. Moreover, similar recoveries were obtained after the clean-up step, Table 4. Thus, compounds are quantitatively eluted from the silica cartridge using 10 ml of ethyl acetate (this volume can be reduced to 5 ml if estriol is not considered), and non significant analytes losses are produced in the further evaporation of this extract to 0.1 ml.

Recoveries for spiked river (2000 ml) and sewage water (1000 ml) are also given in Table 4. Results were similar to those obtained for spiked Milli-Q water. In case of river water the derivatized estrogens could be quantified by GC–MS without the need of the clean-up step. However, for the sewage samples a yellowish extract was obtained, therefore a clean-up step was mandatory. Furthermore, in case of influent samples, even after the clean-up step using a silica cartridge, the high levels of interferences observed in the earlier region of the GC–MS chromatograms, prevented the quantification of both species, Fig. 3. The use of alumina or florisil, as alternatives to silica in the clean-up step, led to similar chromatographic profiles, while an even higher level of interferences was found using cianpropyl or amino sorbents (figure not shown). Therefore, samples from the inlet stream of the sewage treatment plant were first submitted to the clean-up step (silica remained as the clean-up sorbent) and then the organic extract analysed using MS–MS detection. In both influent and effluent samples, recoveries given in Table 4 were obtained after the subtraction of peak areas in blank (non-spiked samples). The excellent agreement between the recoveries obtained for 17α-ethinylestradiol, Table 4, using MS (effluent) and MS–MS detection (influent) suggested that this compound did not co-elute with other species, presenting the same quantification ion in the MS spectra, as it has been suggested in the literature. [2,18].

3.3. Performance of the analytical method

Linearity of the method was tested with standard mixtures (previously derivatized under the optimal conditions) containing 17β-estradiol-d_4 as internal standard (250 ng/ml), and increasing concentrations of the estrogens, at seven concentration levels, between the quantification limits and 1000 ng/ml. Using both MS and MS–MS detection correlation coefficients higher than 0.993 were obtained for all compounds. Relative standard deviations for 5 consecutive injections of a standard, containing all species at the 150 ng/ml level, ranged from 1 to 6% using either MS either MS–MS detection, Table 5. Obviously, the better repeatability and the higher correlation coefficients always corresponded to the 17β-estradiol, since any variation in the instrumental response for this compound is effectively compensated by the used internal standard. Quantification limits of the analytical procedure for sewage water, using MS and MS–MS detection, were evaluated taking in account the instrumental quan-

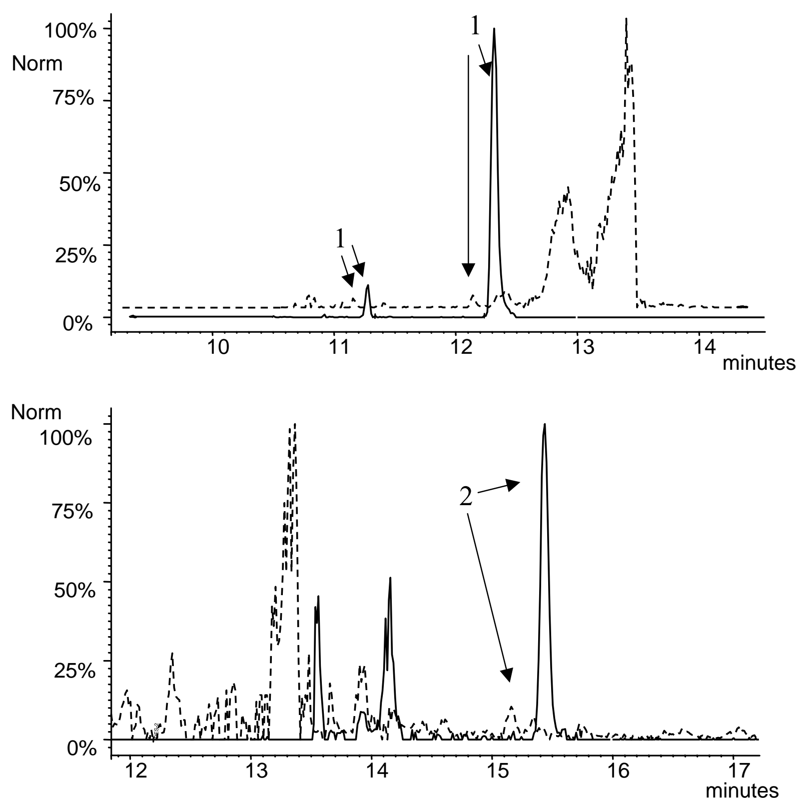

Fig. 3. Normalized GC–MS (dotted line) and GC–MS–MS (solid line) chromatograms for a spiked (75 ng/l) influent sewage water sample after concentration at pH 6 and clean-up using a silica cartridge. Compounds: (1) diethylstilbestrol (*m/z*: 412, MS; *m/z*: 383, MS–MS); (2) estrone (*m/z* 342, MS; *m/z*: 257 MS–MS). Retention times in the MS–MS chromatogram have been displaced to facilitate peak observation.

tification limits (for an injected volume of 2 μl) and the enrichment factors obtained in the extraction process, Table 5. However, it should be noted that using the proposed sample preparation procedure, quantification of estrogens in influent of sewage water treatment plants must be accomplished by GC–MS–MS and not by single GC–MS, due to the complexity of this kind of samples. For river water, quantification limits are twice lower than those given in Table 5, when 2000 ml of sample are considered. Moreover, the use of large volume injectors will allow a further improvement in the sensitivity of the method for relatively clean samples as river water.

3.4. Analysis of real samples

The developed procedure was applied to the analysis of estrogens in a sewage water plant equipped with primary and biological (activated sludge) sample treatments. Discrete water samples were simultaneously taken in the inlet and outlet streams of the plant and processed immediately. Analysis were performed using MS (only effluent) and MS–MS detection (for both influent and effluent). Mestranol, ethinylestradiol and diethylstilbestrol were not found in both samples; however, relative high concentrations of the natural estrogens were measured, Table 6. Fig. 4

Table 5
Linearity, repeatability and quantification limits (QL) for the proposed method

Compound	Correlation coefficient (R^2)		Repeatability ($n = 5$; R.S.D., %)		Instrumental QL ($S/N = 10$), (ng/ml)		Method QL ($S/N = 10$), ng/l	
	MS	MS–MS	MS	MS–MS	MS	MS–MS	MS	MS–MS
Diethylstilbestrol	0.996	0.996	3.3	2.5	7	3	2	1
Estrone	0.997	0.993	4.2	3.2	10	3	3	1
17β-Estradiol	0.999	0.998	1.5	1.6	10	7	3	2
Mestranol	0.999	0.995	2.5	5.8	20	10	6	3
17α-Ethinylestradiol	0.999	0.995	2.6	2.6	17	10	5	3
Estriol	0.998	0.996	2.4	6.0	20	10	6	3

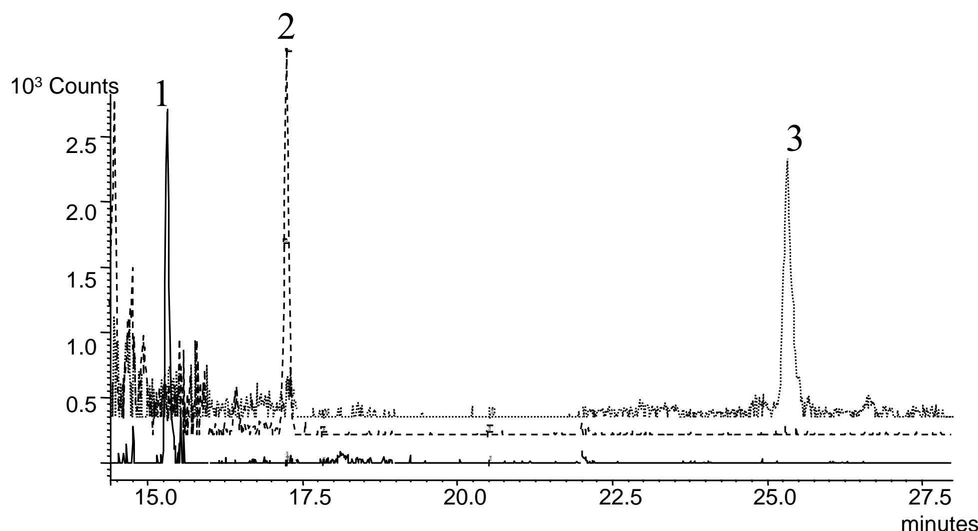

Fig. 4. GC–MS–MS peaks of estrone (1), 17β-estradiol (2) and estriol (3) in a non-spiked sewage water (influent). Plotted chromatograms correspond to quantification ions given in Table 1.

Table 6
Levels of estrone, 17β-estradiol and estriol in the influent and effluent of a sewage water treatment plant ($n = 3$ replicates)

Compound	Mean value (ng/l) ± S.D.		
	Influent, MS–MS detection	Effluent, MS–MS detection	Effluent, MS detection
Estrone	30.8 ± 0.7	33 ± 3	30 ± 4
17β-Estradiol	13.1 ± 0.8	2.9 ± 0.1	3.3 ± 0.5
Estriol	62 ± 10	<QL	<QL

corresponds to the GC–MS–MS signals of estrone, 17-βestradiol and estriol in the influent of the wastewater treatment plant using quantification m/z ratios given in Table 1. Found concentrations, Table 4, suggested that estriol was completely removed from the aqueous stream in the water treatment plant, while removal efficiency for 17β-estradiol was found to be about 75%, and the levels of estrone in the influent and effluent of the plant were equivalents. In case of estrone and 17β-estradiol, these removal yields are similar to those previously published for a treatment plant in Germany (estriol was not included in that study) [2].

4. Conclusions

An analytical procedure for the determination of six estrogens in water by SPE and GC–MS or GC–MS–MS has been developed. After optimization of the derivatization conditions it was proved that MSTFA, without the use of any catalyser, lead to the complete silylation of all the –OH groups of these compounds. The proposed method allows the simultaneous determination of six estrogens in water samples without pH adjustment by using commercial SPE cartridges for extraction and clean-up, avoiding the packing step of the sorbents into glass columns often found in the literature. The limits of quantification obtained (2–6 ng/l with MS and 1–3 ng/l with MS–MS detection) allowed the application of the developed method to the determination of estrogens in samples from a sewage treatment plant. Only the presence of natural estrogenic compounds (estrone, estradiol and estriol) in concentrations up to 62.3 ng/l (estriol) was noticed in the influent. Estriol was completely removed during water treatment; however, estradiol was only partially removed and estrone was not eliminated at all.

Acknowledgements

Financial support from the Spanish MCYT-DGI (project BQU 2003-02090) is acknowledged. J.C. and J.B.Q. acknowledge their doctoral grants from the regional government (Xunta de Galicia) and the Spanish Ministry of Education, respectively. Aquagest is thanked for the supply of sewage water samples.

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