Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1056 (2004) 179–185
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Applicability of solid-phase microextraction followed by on-fiber silylation for the determination of estrogens in water samples by gas chromatography–tandem mass spectrometry

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

Departamento de Química Analítica, Nutrición y Bromatología, Instituto de Investigación y Análisis Alimentario, Universidad de Santiago de Compostela, Santiago de Compostela 15782, Spain

Available online 28 July 2004

Abstract

A solvent-free method for the determination of five estrogens in water samples at the low ng/l was optimized. Compounds were first concentrated on a polyacrylate (PA) solid-phase microextraction (SPME) fiber, directly exposed to the water sample, and then on-fiber silylated on the headspace of a vial containing 50 μl of *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA). Derivatized analytes were determined using GC with MS/MS detection. Influence of several factors on the efficiency of the microextraction step (e.g. time, sample volume, pH, ionic strength and fiber coating) is systematically described. Derivatization conditions were optimized in order to achieve the complete silylation of all hydroxyl groups contained in the structure of the compounds. Detection limits (from 0.2 to 3 ng/l) are compared with those obtained using the same detection technique and different sample preparation strategies, such as solid-phase extraction followed by silylation of the analytes in the organic extract and SPME without derivatization. The method was applied to the analysis of sewage water samples. Two of the investigated species were detected above the quantification limits of the procedure.

Keywords: Water analysis; Derivatization, GC; Solid-phase microextraction; On-fiber derivatization; Estrogens

1. Introduction

Since the discovery of their strong activity as endocrine disrupter chemicals estrogens are considered as target compounds in the aquatic environment. Urinary excretion of estrogenic hormones, together with their de-conjugation and incomplete removal in waste water treatment plants, constitutes the main input of these species in the aquatic environment. Compounds such as estrone, 17β-estradiol and 17α-ethinylestradiol have been detected at the low ng and sub-ng/l in waste and surface waters [1–3]. Even at this low concentrations, they affect the normal reproduction of aquatic organisms showing a stronger estrogenic activity than other well known chemical pollutants such as nonylphenols and PCBs [4]. Effects of other hormones, e.g. diethylstilbestrol used as a fraudulent grow promoter in the feed of farm animals, on aquatic organisms have been scarcely studied.

Most procedures applied to the determination of estrogens in water are based on the concentration of large samples (from 0.5 to 2 l), followed by their selective detection using GC or LC in combination with MS, or preferably tandem MS spectrometry. GC based methods require the derivatization of the hydroxyl groups contained in the structure of the analytes in order to improve the sensitivity of the method. Acetylation and silylation reagents have been considered for this purpose [5–7]. In general, the aliphatic –OH groups show a lower reactivity than the aromatic ones, and thus they are more difficult to derivatize; particularly, the sterical hindered hydroxyl groups in position 17 of mestranol and 17α-ethinylestradiol [8–9]. From our knowledge, the only compound able to react quantitatively with all –OH groups of both species is the *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA) [10]. LC-MS methods do not require the derivatization of the analytes; however, the ionization efficiency can be affected by changes in the composition of the mobile phase and moreover, co-extracted interferences may produce signal suppression problems [11].

* Corresponding author. Fax: +34 981 595012.
E-mail address: qnisaac@usc.es (I. Rodríguez).

0021-9673/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2004.06.111

Traditionally, solid-phase extraction (SPE), using different reverse phase materials [3–4,12–13], has been the preferred technique for the extraction of estrogens from water samples. After the concentration step, organic extracts are usually purified using normal phase sorbents or liquid chromatography fractionation, previously to the analytical determination of the compounds [3–4,14].

SPME has also been successfully employed for the determination of polar and non-polar pollutants in water samples. In comparison to SPE protocols, less sample volume is required and generally, a higher selectivity is achieved. Peñalver et al. [15], using HPLC as the separation technique, have demonstrated the possibilities of polar SPME fibers to extract estrogens from water samples. Braun et al. [16] have proposed the combination of SPME with GC-MS for the determination of several endocrine disrupters (including 17α-ethinylestradiol) in water samples; however, as compounds were not derivatized, relatively high quantification limits were obtained. Reagents used for the silylation of estrogenic hormones are destroyed in presence of water, therefore, microextraction and derivatization steps cannot be performed simultaneously. The problem can be solved by dividing the procedure in two steps: compounds are first incorporated on the fiber and then on-fiber silylated in a dry atmosphere. This approach has been successfully applied to the analysis of different groups of polar organic compounds in water samples [17–19]. In fact, a few years ago, Okeyo et al. [20] have already demonstrated the possibility of performing the derivatization of estrone and 17β-estradiol in a SPME fiber using bis-(trimethylsilyl) trifluoroacetamide (BSTFA). However, their work was mainly focused in biological samples, and thus both compounds were determined at high concentration levels.

The objective of this study is to evaluate the possibility of combining SPME, followed by on-fiber derivatization, and gas chromatography for the determination of several natural and synthetic estrogenic hormones in water samples at the low ng/l level. Analytes were first incorporated in a polar SPME fiber and then derivatized using MSTFA. Influence of extraction and derivatization conditions on the efficiency of the whole analytical procedure is systematically studied. Detection limits are compared with those obtained after solid-phase concentration of large sample volumes, purification of this extract and silylation of the analytes with the same reagent.

2. Experimental

2.1. Standards, reagents and samples

Standards of diethylstilbestrol (DES), estrone (E1), 17β-estradiol (E2), 17α-ethinylestradiol (EE2) and mestranol (MES) were obtained from Aldrich (Milwaukee, WI, USA). Deuterated 17β-estradiol (d_4), ($E2d_4$), deuterium was introduced in positions 2, 4 and 16, was purchased from Cambridge Isotope Laboratories (Andover, MA, USA). Diluted standards and mixtures of them were made in methanol. Individual solutions of $E2d_4$ were also prepared in methanol. This compound was normally used as internal standard throughout the analytical procedure. The derivatization reagent *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA) was also purchased from Aldrich in 1 ml ampoules.

A manual SPME holder and fibers with different coatings: poly(dimethylsiloxane) (PDMS, 100 μm film thickness), polyacrylate (PA, 85 μm film thickness), poly (dimethylsiloxane-divinilbenzene) (PDMS-DVB, 65 μm film thickness), carboxen-PDMS (CAR-PDMS, 75 μm film thickness) and Carbowax-DVB (CW-DVB, 75 μm film thickness), were obtained from Supelco (Bellefonte, PA, USA).

Spiked and non-spiked water samples (Milli-Q, river and waste water) were used in this study. Waste water samples were collected in the influent and the effluent of an urban sewage plant equipped with primary and secondary treatments. Samples were filtered at 0.45 μm when received and then stored at 4 °C until being analyzed.

2.2. Equipment

Determination of the derivatized analytes was carried out by GC-MS or GC-MS/MS using a Varian CP-3800 CX gas chromatograph (Walnut Creek, CA, USA) equipped with a split–splitless injection port and connected to an ion-trap mass spectrometer (Varian Saturn 2000). Chromatographic separations were achieved using a BP-5 type capillary column (30 m × 0.25 mm i.d., d_f: 0.25 μm) obtained from Varian. Helium was used as carrier gas at a constant flow of 1.0 ml/min. The GC oven was programmed as follows: 5 min at 50 °C, first ramp at 20 °C/min to 220 °C (held for 17 min), 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. SPME fibers were desorbed during 5 min, in the splitless mode, using the following temperatures: 250 °C for PDMS and PDMS-DVB, 280 °C for PA and CAR-PDMS, and 220 °C for CW-DVB coated fibers. Mass spectra were obtained using the electron impact ionization mode (70 eV). MS/MS fragmentation conditions were optimized in a previous paper [10]. *m*/*z* ratios used for quantification of the completely silylated analytes were 412 (DES), 342 (E1), 416 (E2), 420 ($E2d_4$), 425 (EE2), and 367 (MES), using MS detection and 383 (DES), 257 (E1), 285 + 326 (E2), 287 + 330 ($E2d_4$), and 193 (EE2, MES) for MS/MS detection.

2.3. Sample preparation

Samples (100 ml) were adjusted to pH 6, spiked with the internal surrogate $E2d_4$, and placed in 115 ml glass vials containing a stir bar and 300 mg of sodium chloride per ml of water. The vial was closed and a PA fiber was dipped into the sample. After finishing the microextraction step, it was exposed to the headspace of a 1.5 ml vial containing 50 μl of

MSTFA. On-fiber derivatization was carried out at 60 °C for 30 min.

Concentrations of the analytes in spiked and non-spiked samples were normally determined with the standard addition procedure. The ratio analyte peak area/E2d_4 peak area was plotted versus the concentration of each compound added to the microextraction vessel. Additionally, the possibility of quantifying E2 in sewage water samples using the isotopic dilution technique was also considered.

3. Results and discussion

3.1. Evaluation of on-fiber derivatization conditions

Initial experiments were performed using a PA fiber. After its exposition to water samples, spiked with the estrogenic hormones, the influence of derivatization time (from 10 to 60 min) and temperature (from 40 to 80 °C) on the yield of the on-fiber silylation was investigated using GC-MS detection. In all cases 50 μl of MSTFA were employed. The behavior of the analytes followed two different patterns. Peak areas corresponding to the silyl derivatives of DES, E1 and E2 were scarcely affected by the operating conditions. As shown for E2 (Fig. 1A), only in case of long derivatization times using a high temperature (80 °C) a significant decrease in the peak area of the silylated compound was observed. Probably, under these conditions E2 was partially desorbed from the PA fiber. Extra peaks corresponding to non-silylated (E1) or mono-silylated species (for DES and E2) were not observed, figure not shown. However, in the case of DES two peaks with the same mass spectra, corresponding to the *cis* and *trans* isomers, were obtained [10–11].

In contrast to this behavior, the yield of the on-fiber derivatization for EE2 and MES was heavily affected by time and temperature conditions. Short exposition times at low temperatures led to the incomplete silylation of both analytes. In these conditions, two peaks were obtained for each compound. In the case of MES they corresponded to the derivatized (base peak *m*/*z* 367) and non-derivatized compound (base peak *m*/*z* 310). For EE2 one peak corresponded to the silylation of both hydroxyl groups contained in its structure (base peak *m*/*z* 425) and the other one to the mono-silylated specie (base peak *m*/*z* 368). The increase in the derivatization time and temperature produced a diminution in the peak area of the monosilylated EE2 (Fig. 1B), at the same time that the response for the fully derivatized (di-silylated) compound raised (Fig. 1C). The same behavior was observed for MES, figure not shown. Optimal on-fibre silylation time and temperature were fixed at 30 min and 60 °C, respectively. Variations in the volume of MSTFA, from 20 to 100 μl, did not affect the yield of the derivatization; thus, it was maintained in a medium value (50 μl). Under optimal silylation conditions, peak areas for the non-derivatized MES and the mono-derivatized EE2 represented less than 2–3% of those corresponding to the silylated compounds.

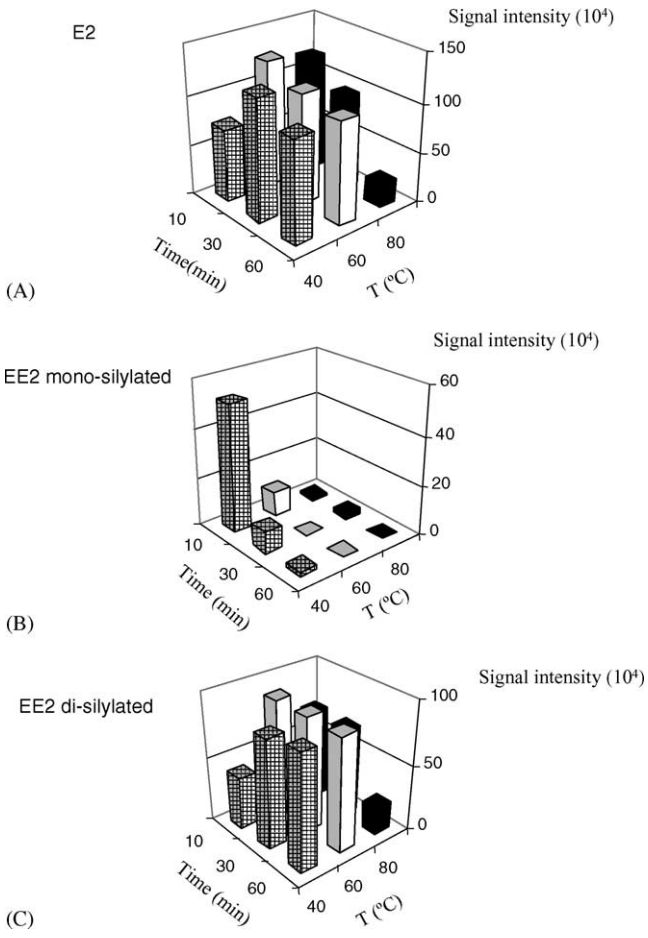

Fig. 1. Influence of derivatization time and temperature on the efficiency of on-fibre silylation. A 50 μl of MSTFA were used in all experiments. (A) Plot of peak areas for E2 as silylated compound. (B and C) Plots of peak areas for EE2 as mono and di-silylated derivatives, respectively.

3.2. Optimization of microextraction conditions

3.2.1. Fiber coating

The effect of the fiber coating on the yield of the extraction was evaluated using spiked Milli-Q water samples (ca. 500 ng/l). The extraction time was fixed at 40 min and derivatization was performed using conditions optimized for the PA fiber. In all cases only one peak (with the exception of DES) was obtained per compound. The highest responses were achieved using the polar PA fiber. The extraction efficiency of the PDMS-DVB one was a 70% lower and, globally, even poorer results were achieved with the CAR-PDMS and the PDMS fibers. The efficiency of the CW-DVB fiber could not be evaluated since it resulted extremely unstable when submitted to the proposed method. After two or three extraction–derivatization cycles the coated phase became detached from the silica core.

3.2.2. Stirring, pH and sodium chloride

Influence of these factors on the yield of the microextraction was evaluated using a factorial experimental design at

Table 1
Experimental domain and standardized main effects for the factors considered in the experimental factorial design

Factor	Low level	High level	Standardized main effect				
			DES	E1	E2	MES	EE2
pH	4	8	−2.3	−0.36	−0.32	−3.08	−1.39
NaCl (mg/ml)	0	300	4.76[a]	6.69[a]	6.29[a]	−2.20	3.21[a]
Stirring	Without	With	9.74[a]	8.61[a]	8.80[a]	12.08[a]	8.31[a]

[a] Statistically significant factors at the 95% confidence level.

two levels (2^3) with two central points. Low and high levels for those factors, together with their normalized main effects are given in Table 1. Stirring was the most important factor with a significant and positive effect on the yield of the microextraction, especially for the samples spiked with sodium chloride. The addition of sodium chloride also showed a positive and significant influence on the microextraction efficiency, except in the case of MES. Regarding sample pH, the highest efficiency was achieved at pH 4; however, the effect of this factor was not statistically significant (95% confidence level) (Table 1). Addition of sodium chloride (300 mg/ml), pH 6 and magnetic stirring (400 rpm) were fixed as working conditions. After extraction, and previously to the silylation step, the fiber was exposed during 2 min to Milli-Q water in order to remove the excess of sodium chloride from its surface.

3.2.3. Sample volume

The influence of the sample volume on the amount of each compound concentrated on the PA fiber was evaluated using vials with capacities of 10, 20, and 115 ml. Considering an extraction time of 30 min, twice higher signals (peak areas) were obtained for the 115 ml vessels than for the 10 ml ones. Therefore, 100 ml samples (containing 30 g of NaCl) were employed in further experiments.

3.2.4. Sampling and desorption time

The effect of sampling time in the yield of the microextraction is shown in Fig. 2. In agreement with other published results [16] the kinetic of the extraction was very slow, especially for MES and EE2, which presented equilibrium times longer than 2 h. Despite of these results, the microextraction time was adjusted to 60 min in order to limit the duration of the sample preparation step. Memory effects in the PA fiber, after a desorption step of 5 min at 280 °C, were investigated in order to detect the presence of derivatized and non-derivatized species in the coating. In the first case, the fiber was desorbed twice after an extraction–derivatization cycle. In the second one, after a first desorption, the fiber was exposed again to a fresh aliquot of the silylation reagent. In both situations between 2 and 5% of the compounds were detected in the second fiber desorption; therefore, it seems that memory effects are mainly due to silylated species. To avoid contamination problems during the analysis of samples containing different concentrations of the estrogenic compounds, fibers were additionally heated at 280 °C for 5 min between consecutive injections.

3.2.5. Performance of the analytical procedure

The linearity of the method was investigated using Milli-Q water samples spiked with the estrogenic hormones at five concentration levels from 5 to 500 ng/l. $E2d_4$ was also added to all samples at 25 ng/l and used as internal surrogate. Correlation coefficients (r^2) from 0.994 to 0.999 were obtained (Table 2). Relative standard deviations of the method ranged from 1 to 12% (Table 2). Quantification limits from 0.2 to 3 ng/l were achieved for all species using MS/MS detection. These values are similar than those previously reported by our group after the solid-phase extraction of 1 l samples [10], and 20 times lower than the value reported for EE2 by Braun et al. [16] using a PA fiber but, without introducing the on-fiber derivatization step.

Accuracy was estimated using river water spiked with the analytes at 25 ng/l. Samples were stored overnight at 4 °C and the concentration of each hormone determined using the standard addition method. Found values ranged from 80 to 120%

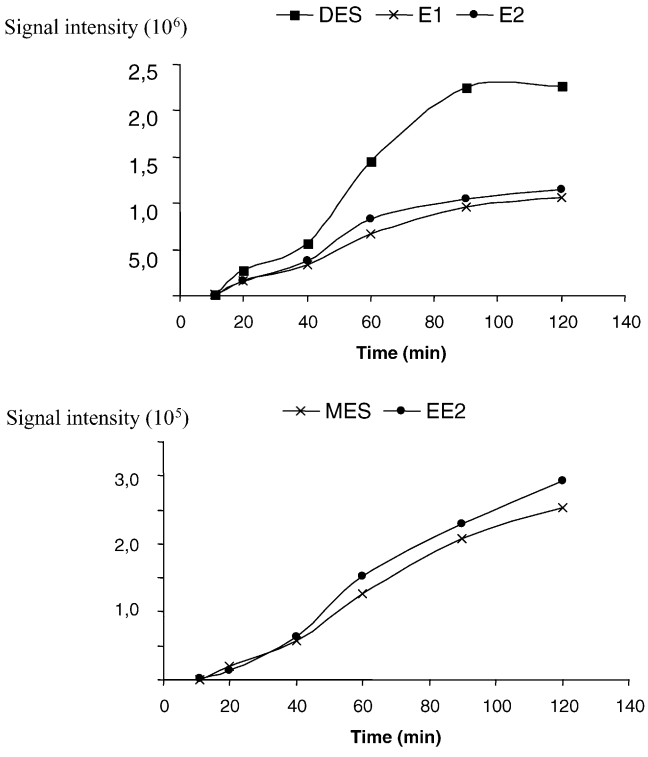

Fig. 2. Kinetic curves for the direct microextraction of estrogens using a PA fiber. Samples were adjusted to pH 6 and 300 mg of sodium chloride per ml added.

Table 2
Linearity, repeatability, quantification limits and accuracy of the proposed method considering an extraction step of 60 min, 100 ml samples, and using MS/MS detection

Compound	Linearity correlation coefficient (r^2)	Repeatability (n = 4 replicates)			Q.L. (S/N = 10) (ng/l) GC-MS/MS			Accuracy (river water)	
		R.S.D.% spiked concentration			SPME + silylation	SPE (1 l), clean-up, silylation	SPME without derivatives[a]	Added concentration (ng/l)	Found concentration (ng/l) (mean ± S.D.)
		500 ng/l	50 ng/l	25 ng/l	This work	Ref. [10]	Ref. [16]		
DES	0.998	3	9	10	0.2	1	–	27.1	28 ± 3
E1	0.994	8	5	1	1	1	–	25.1	31 ± 1
E2	0.998	5	4	8	0.7	2	–	25.9	27 ± 2
MES	0.999	3	7	12	3	3	–	25.2	20 ± 2
EE2	0.999	6	9	5	3	3	60	25.8	23 ± 1

[a] GC-MS detection.

of the spiked concentrations with relative standard deviations around 10% (Table 2).

Matrix effects were investigated using different water samples previously adjusted at pH 6 and filtered. After addition of the analytes (1 ng/ml), they were immediately submitted to extraction–derivatization procedure (n = 3 replicates). Similar responses were obtained for all compounds in case of ultrapure and river water; however, matrix effects were observed for sewage water (Fig. 3). In the case of E1, E2 and EE2, the most polar compounds (log K_{ow} 3.7, 4.1 and 4.5, respectively) a moderate decrease in the yield of the process was observed. For DES (log K_{ow} 5.9) and MES (log K_{ow} 5.2), a dramatic decay was observed, especially for non-treated waste water. Probably, in presence of a high amount of dissolved organic matter, the diminution of the partition coefficients between the fiber and the sample (k_{fs}) is most acute for the less polar compounds.

3.3. Analysis of real samples

The method was applied to the determination of estrogenic hormones in treated sewage waters. Two samples were taken in September (S1) and December 2003 (S2). After reception they were adjusted at pH 6, filtered, spiked with a solution of E2d$_4$ and analyzed in the same day (Fig. 4). Estrone and 17β-estradiol were found in both samples above the quantification limits of the proposed method. Found concentrations (17.3 ± 0.9, S1; 8.1 ±1.1, S2, ng/l for E1 and 7.6 ± 1.6, S1, 5.0 ± 0.5, S2, ng/l for E2) are similar to those previously reported for the same treatment plant, using a different sample preparation strategy [10].

3.4. Quantification of 17β-estradiol using isotopic dilution

Efficiency of SPME methods can be affected by matrix effects (Fig. 3) and the by the age of the fiber; therefore, proper quantification should performed using the time consuming standard addition technique. Thus, in addition to the replicates of the original sample, different aliquots should be spiked with growing concentration of the analytes and submitted to the microextraction procedure. The isotopic dilution (ID) technique avoids the preparation of spiked samples and improves the precision and the accuracy of the results. In combination with GC-ICP MS, ID has been already successfully applied to the determination of labile organometallic species which can be partially decomposed during the sample preparation step [21]. However, in the field of organic analysis, using MS or MS/MS detection with electronic impact ionization, the use of ID is not very frequent.

In this section of the article, ID was used to quantify the concentration of E2 in the above considered sewage water samples (S1 and S2). In order to determine the purity of deuterated estradiol and also to investigate if completely independent detection channels could be achieved for both species, silylated individual standards of E2 and E2d$_4$

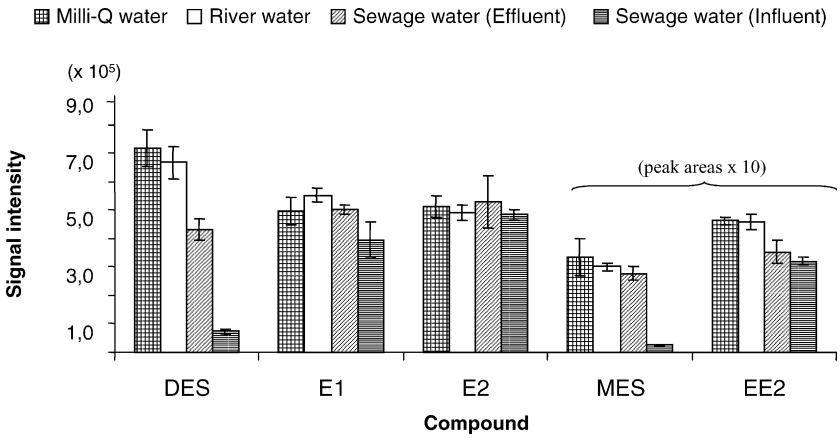

Fig. 3. Influence of the water matrix on the yield of the microextraction. Samples were spiked (1 ng/ml level) after filtration and previously to the microextraction step. Signals (peak areas) for MES and EE2 were multiplied by a factor of 10, to obtain the same scale for all compounds.

(500 ng/ml), were prepared in ethyl acetate [10] and analyzed using MS/MS detection. Parent ions 416 (E2) and 420 ($E2d_4$) were isolated with a window of ±1 *m/z* unit. E2 and $E2d_4$ signals were integrated using the *m/z* ratios of 326 and 330, respectively. In the case of $E2d_4$, no signal was detected when the chromatogram at 326 *m/z* was monitored. For the native estradiol (E2) the signal recorded at 330 *m/z* represented around 0.2% of the peak area at 326 *m/z*. It was assumed that the E2 response at 330 *m/z* was the result of the natural abundance of the ^{13}C isotope (around 1%) and the limited capability of the mass spectrometer to isolate the E2 parent ion (*m/z* 416). Anyhow, unless the concentration of the native estradiol in the sample was much higher than that of the deuterated compound, the contribution of this interference to the final result was small and it was not considered for further calculations. Therefore, assuming that the response of the GC-MS/MS system is the same for the native and the deuterated 17β-estradiol, the concentration of the analyte in the sample could be calculated using the following simplified equation:

$$E2\text{conc (ng/l)} = \frac{\text{Peakarea}(326\,m/z)}{\text{Peakarea}(330\,m/z)}\frac{272.4}{276.4}E2d_4\ \text{conc(ng/l)}$$

Being 272.4 and 276.4 the molecular weights of E2 and $E2d_4$, respectively; and $E2d_4$conc the concentration of the deuterated 17β-estradiol in the SPME vial.

Measured E2 concentrations, 7.85 ± 0.05 ng/l (S1) and 5.34 ± 0.09 ng/l (S2), showed an excellent agreement with those obtained using the standard addition method (Section 3.4). The best precision, and the smallest experiment effort, were obtained by ID. Obviously, the validity of the above proposed simplified equation should be tested for the rest estrogens using their corresponding isotopic labeled species. Theoretically, the bigger the difference between *m/z* ratios of the parent ions for native and labeled species, the lower the possibility to found cross contributions

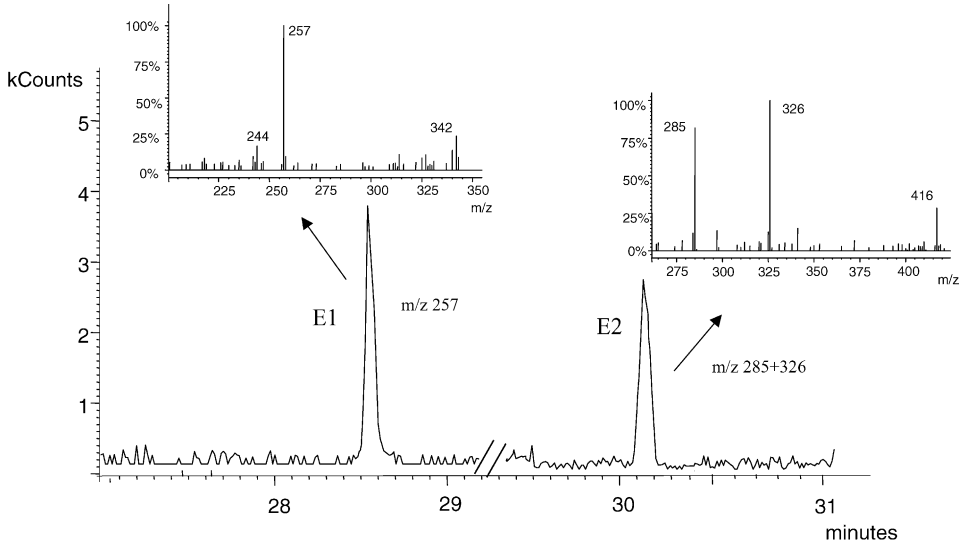

Fig. 4. GC-MS/MS chromatogram showing the peaks of E1 (8.1 ± 1.1 ng/l) and E2 (5.0 ± 0.5 ng/l) in a real, non-spiked sewage water sample.

to the peak areas of their daughter ions in the MS/MS chromatograms.

4. Conclusions

An alternative SPME sample preparation method for the determination of five estrogenic hormones in water samples has been developed. The inclusion of the on-fiber derivatization step provided a considerable improvement on the achieved sensitivity in comparison to previously published SPME methods. Moreover, quantification limits are similar to those reported for SPE methods. The study of matrix effects demonstrated that the method is applicable for the determination of all the compounds in surface and sewage water, with the exception of DES and MES in non-treated sewage water.

Considering E2 as a model compound and using GC with MS/MS detection, isotopic dilution analysis has proved to be an attractive alternative to the standard addition technique, improving the precision of the results and reducing the experimental effort. Simplified isotopic dilution equations can be used under the condition that completely independent channels were obtained for MS/MS detection of daughter ions from the native and the labeled species.

Acknowledgments

This work has been financially supported by the Spanish DGICT (project BQU 2003-02090). J. Carpinteiro and J.B. Quintana acknowledge their doctoral grants from the regional government (Xunta de Galicia) and the Spanish Ministry of Education, respectively.

References

[1] C.G. Daughton, T.A. Ternes, Environ. Health Perspect. 107 (1999) 907.
[2] K. Kümmerer, Pharmaceuticals in the Environment, Springer, Berlin, 2001.
[3] G. Ascenzo, A. Di Corcia, A. Gentili, R. Manzini, R. Mastropasqua, M. Nazzari, R. Samperi, Sci. Total Environ. 302 (2003) 199.
[4] T.A. Ternes, M. Stumpf, J. Mueller, K. Haberer, R.D. Wilken, M. Servos, Sci. Total Environ. 225 (1999) 81.
[5] C. Kelly, J. Chromatogr. A 872 (2000) 309.
[6] T.R. Croley, R.J. Hughes, B.G. Koenig, C.D. Metcalfe, R.E. March, Rapid Commun. Mass Spectrom. 14 (2000) 1087.
[7] M.J. López de Alda, D. Barceló, J. Chromatogr. 371 (2001) 437.
[8] C. Huang, D.L. Sedlak, Environ. Toxicol. Chem. 20 (2001) 133.
[9] O. Lerch, P. Zinn, J. Chromatogr. A 991 (2003) 77.
[10] J.B. Quintana, J. Carpinteiro, I. Rodríguez, R.A. Lorenzo, A.M. Carro, R. Cela, J. Chromatogr. A 1024 (2004) 177.
[11] T. Benijts, R. Dams, W. Günter, W. Lambert, A. De Leenheer, Rapid Commun. Mass Spectrom. 16 (2002) 1358.
[12] M.J. López de Alda, D. Barceló, J. Chromatogr. A 911 (2001) 203.
[13] H.M. Kuch, K. Ballschmiter, Fresenius J. Anal. Chem. 366 (2000) 392.
[14] A.C. Belfroid, A. Van der Horst, A.D. Vethaak, A.J. Schäfer, G.B.J. Rijs, J. Wegener, W.P. Cofino, Sci. Total Environ. 225 (1999) 101.
[15] A. Peñalver, E. Pocurrull, F. Borrull, R.M. Marcé, J. Chromatogr. A 964 (2002) 153.
[16] P. Braun, M. Moeder, St. Schrader, P. Popp, P. Kuschk, W. Engewald, J. Chromatogr. A 988 (2003) 41.
[17] I. Rodríguez, J. Carpinteiro, J.B. Quintana, A.M. Carro, R.A. Lorenzo, R. Cela, J. Chromatogr. A 1024 (2004) 1.
[18] R. Alzaga, A. Pena, J.M. Bayona, J. Sep. Sci. 26 (2003) 87.
[19] M.R. Lee, R.J. Lee, Y.W. Lin, C.M. Chen, B.H. Hwang, Anal. Chem. 70 (1998) 1963.
[20] P. Okeyo, S.M. Rentz, N.H. Snow, J. High Resol. Chromatogr. 20 (1997) 171.
[21] Y. Yang, Z. Mester, R.E. Sturgeon, JAAS 17 (2002) 944.