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Journal of Chromatography A, 919 (2001) 275–282

JOURNAL OF CHROMATOGRAPHY A

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Determination of estrogens in river water by gas chromatography–negative-ion chemical-ionization mass spectrometry

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Received 7 November 2000; received in revised form 28 March 2001; accepted 6 April 2001

Abstract

A method for the determination of estrogens (17α-estradiol, 17β-estradiol, estrone, ethynyl estradiol, and estriol) as pentafluorobenzyl-trimethylsilyl (PFB–TMS) derivatives by gas chromatography–mass spectrometry (GC–MS) with negative-ion chemical-ionization (NICI) is described. The NICI of all the derivatives produced an intense $[M-PFB]^-$ ion as the base peak. The reagent gas (methane) flow-rate and the ion source temperature were determined to be 2.0 ml/min and 240°C, respectively, for the optimized NICI-selected ion monitoring (SIM) conditions. The sensitivities of the PFB–TMS derivatives in the NICI mode were 8.0–130 times higher than those of the PFB–TMS derivatives in electron ionization (EI) mode, and 12–25 times higher than those of all the TMS derivatives in the EI mode. This method was applied to the analysis of estrogens in river water using a solid-phase extraction as the sample preparation. The recoveries of the target chemicals from a river-water sample spiked with standards at 2 ng/l level were 85.8–126.5% (RSD, 6.2–13.0%). The methodical detection limits ranged from 0.10 to 0.28 ng/l.

Keywords: Water analysis; Estrogens

1. Introduction

There has lately been a growing interest in chemicals that might be disrupting the endocrine system of humans and wildlife. A variety of chemicals are known to disrupt the endocrine systems of animals in laboratory studies. The endocrine systems of certain wildlife have been affected by chemical contaminants [1,2]. The female hormone (estrogen) such as 17β-estradiol, estrone, ethynyl estradiol, 17α-estradiol, and estriol is considered to be one of the endocrine disrupting chemicals. Estrogen in the environment is derived from the excreta of humans and livestock, medicines, and so on. Since 17β-estradiol has the greatest estrogenic activity and seems to have been discharged into the environment in large quantities, this chemical has been monitored in an endocrine disruptor survey in Japan. Estrone also has significant estrogenic activity and has been detected at high levels near a sewage plant. Ethynyl estradiol has been used as an oral contraceptive. It is recognized that these chemicals like estrogen at the sub-ng/l level act on certain fish [3]. Thus, we need to analyze estrogens in the environment using a method with high sensitivity. Enzyme linked immunosorbent assay (ELISA) and gas chromatog-

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0021-9673/01/$ – see front matter
PII: S0021-9673(01)00851-2

raphy/mass spectrometry (GC–MS) have been used for the analysis of estrogens in environmental samples after sample preparation. Since most ELISA methods have been developed for clinical samples, the determination of estrogens in environmental samples by ELISA tends to show a higher value with interference by the environmental matrix. As for GC–MS, Desbrow et al. reported estrogenic chemicals in sewage-treatment effluents using GC–MS in the electron ionization (EI) mode [4]. Ternes et al. reported a method for the quantification of estrogens in municipal sewage treatment plants using GC–MS–MS in the EI mode [5]. Kuch et al. also reported the determination of estrogens in the effluents from sewage treatment plants using GC–MS in the EI mode [6]. The GC–EI-MS studies achieved about 0.5 to 2 ng/l methodical detection limits after a 5000-fold concentration or more with a clean-up procedure. Furthermore, LC–MS–MS methods have been reported for the determination of estrogens in the environment [7,8]. On the other hand, electron capture negative-ion chemical-ionization (NICI) provides high sensitivity and selectivity for electrophilic compounds. In recent years, GC–NICI-MS has been applied for the trace level determinations of electrophilic compounds containing halogens, nitro groups, and highly conjugated systems in environmental samples [9–11]. With regard to these compounds, which are not inherently electrophilic, with active groups, the compounds can be analyzed by GC–NICI-MS after preparing derivatives such as the pentafluorobenzyl ethers and pentafluorobenzoate esters [12–14].

In this study, a solid-phase extraction was used for the sample preparation. Estrogens were determined by GC–NICI-MS after the derivatization; phenolic hydroxyl groups of the target chemicals were derivatized with pentafluorobenzyl bromide. Subsequently, the remaining aliphatic hydroxyl groups of the target chemicals except for estrone were derivatized with *N*-trimethylsilylimidazole. Although analytical methods for estrogen detection in biological samples by GC–NICI-MS have been reported [15,16], very few studies on the analysis of estrogens in environmental samples have been performed by GC–NICI-MS [12]. We developed a GC–NICI-MS method for five estrogens, and then used it for the analysis of estrogens in river water.

2. Experimental

2.1. Chemicals

17α-Estradiol, 17β-estradiol, estrone, ethynyl estradiol, estriol, and 17β-estradiol-2,4, 16, 16-d_4 were obtained from Wako (Osaka, Japan). Acetone and hexane, both of reagent grade, were purchased from Merck (Darmstadt, Germany). Stock solutions of the individual estrogens were prepared by diluting each compound to a concentration of 1.0 mg/ml in acetone. Anhydrous potassium carbonate (K_2CO_3) was obtained from Wako, and 2 g of K_2CO_3 was diluted in 20 ml of Milli-Q water. Pentafluorobenzyl bromide (PFBBr) was obtained from GL Sciences (Tokyo, Japan), and 0.25 g of PFBBr was diluted in 5 ml of acetone for each use. *N*-trimethylsilylimidazole (TMSI), *N*,*O*-bis(trimethylsilyl)-trifluoroacetamide (BSTFA), and trimethylchlorosilane (TMCS) were obtained from GL Sciences. Excelpak SPE-ENV/124 (Yokogawa Analytical Systems, Tokyo, Japan) was used for solid-phase extraction (SPE).

2.2. Sample preparation

2.2.1. Solid phase extraction

River water was collected from the Tama River (Tokyo, Japan), and was filtered through a 7 μm filter. Four hundred pg of the surrogate (17β-estradiol-2,4,16,16-d_4) was added to the sample (200 ml), and then adjusted to pH 3.5 with 1 *M* hydrochloric acid. The SPE cartridge was washed with acetone (10 ml), and then was conditioned with water (10 ml). The sample (200 ml) was loaded to the cartridge. After the extraction was completed, the cartridge was dried by applying vacuum. The extract was eluted with acetone (3 ml), and then the elute was evaporated to 2 ml with a gentle stream of nitrogen.

2.2.2. Derivatization

One ml of the sample extract was transferred to a 2 ml glass vial (For the determination of instrumental detection limits and of calibration curves, 1 ml of the standard solutions were used for the derivatization). 10% Aqueous potassium carbonate (100 μl) and the acetone solution of 5% PFBBr reagent (100 μl) were added to the vial, and were kept at 60°C for

1 h. After cooling, the solvent was removed to about 100 μl with a gentle stream of nitrogen. Hexane (1 ml) was added, and the organic phase was washed with Milli-Q water (0.5 ml). About 1 ml of the organic phase was transferred to a new 2 ml glass vial, and then the solvent was completely removed with a gentle stream of nitrogen. TMSI (50 μl) was next added to the vial. The vial was kept at room temperature for 30 min. Hexane was added up to 1 ml.

2.3. Instrumentation

All the GC–MS analyses were carried out on an Agilent 6890/5973 (Agilent Technologies, Palo Alto, CA, USA) equipped with an HP-5MS capillary column (30 m×0.25 mm I.D.×0.25 μm film thickness). Helium was used as the carrier gas with a column flow-rate of 1.2 ml/min in constant flow mode, and methane served as the CI reagent gas with a flow-rate of 2.0 ml/min. The GC oven temperature was kept at 150°C for 1.5 min, followed by a ramp up to 310°C at 10°C/min and a final hold for 5 min. The injection port and transfer line temperatures were kept at 260 and 300°C, respectively. The ion source was kept at 240°C. Pulsed splitless injection was used with a pulse pressure of 241 kPa (1.1 min) and a purge time delay of 1.0 min. The MS was operated in the NICI mode and with a scan range of m/z 10 to 700 at 2.17 scans/s. In the selected ion monitoring (SIM) mode, $[M-PFB]^-$ ions and the second largest ions were monitored for all compounds with a dwell time of 40 to 150 ms per single ion. The injection volume was 4.0 μl.

3. Results and discussion

3.1. Derivatization conditions

The phenolic hydroxyl groups of estrogens (10 μg each) were completely derivatized with pentafluorobenzyl bromide by the derivatization method described in the Experimental section. We studied some conditions to optimize the trimethylsilyl derivatization of the remaining aliphatic hydroxyl groups of the estrogens by GC–EI-MS in the scan mode. The studied conditions are as follows: (A) 200 μl of BSTFA was added to the analyte and the vial was heated at 60°C for 0.5 h; (B) 200 μl of BSTFA and 800 μl of hexane were added to the analyte and the vial was heated at 80°C for 1 h; (C) 200 μl of BSTFA containing 1% TMCS was added to the analyte and the vial was heated at 90°C for 1 h; (D) the hexane solution after the pentafluorobenzylation described in the Experimental section was dried over anhydrous sodium sulfate before procedure (C); (E) 50 μl of TMSI was added to the analyte and the vial was kept at room temperature for 0.5 h. Fig. 1 shows the remaining percentage of estrogens with aliphatic hydroxyl groups after the above conditions. The aliphatic hydroxyl groups did not completely react with BSTFA using the derivatization procedures of (A), (B), (C), and (D). However, the aliphatic hydroxyl groups completely reacted with TMSI when using derivatization procedure (E).

3.2. NICI mass spectra of PFB–TMS derivatives and EI mass spectra of PFB–TMS derivatives and all the TMS derivatives

In the NICI mode, the pentafluorobenzyl-tri-

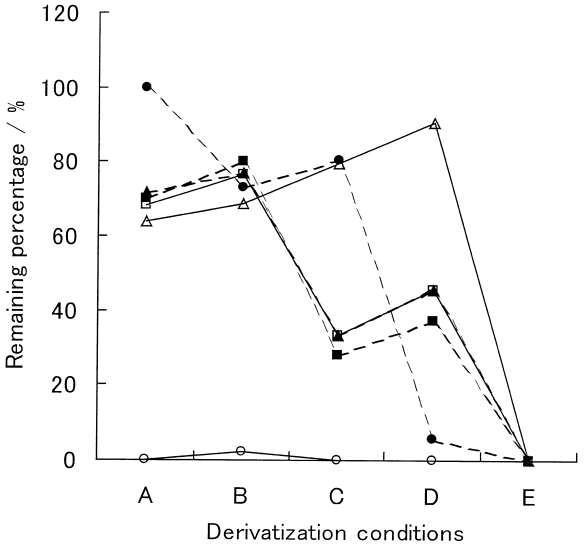

Fig. 1. Remaining percentage of estrogens with aliphatic hydroxyl groups under the different derivatization conditions. ■ 17α-estradiol–PFB; □ 17β-estradiol-2,4,16,16-d_4–PFB; ▲ 17β-estradiol–PFB; △ ethynyl estradiol–PFB; ● estriol–PFB–TMS; ○ estriol–PFB. For the derivatization conditions, see the Results and discussion.

methylsilyl (PFB–TMS) derivatives of the estrogens produced an intense $[M–PFB]^-$ ion using a dissociative electron capture process. The $[M–PFB]^-$ion is of course characteristic of the original estrogens and should be useful for quantification by SIM. In the EI mode, the PFB–TMS derivatives of all the target compounds except for ethynyl estradiol produced the M^+ ion as the major ion. The PFB–TMS derivative of ethynyl estradiol produced the $[M-15]^+$ ion as the major ion. The PFB^+ (m/z 181) ion was observed for all target compounds. The TMS^+ (m/z 73) ion was also observed for all target compounds except for estrone. Next, all the TMS derivatives of all the target compounds produced the M^+ ion as the major ion. The TMS derivative of ethynyl estradiol produced the $[M-15]^+$ ion as the second largest ion. The TMS^+ (m/z 73) ion was observed for all the target compounds. Fig. 2 shows the NICI mass

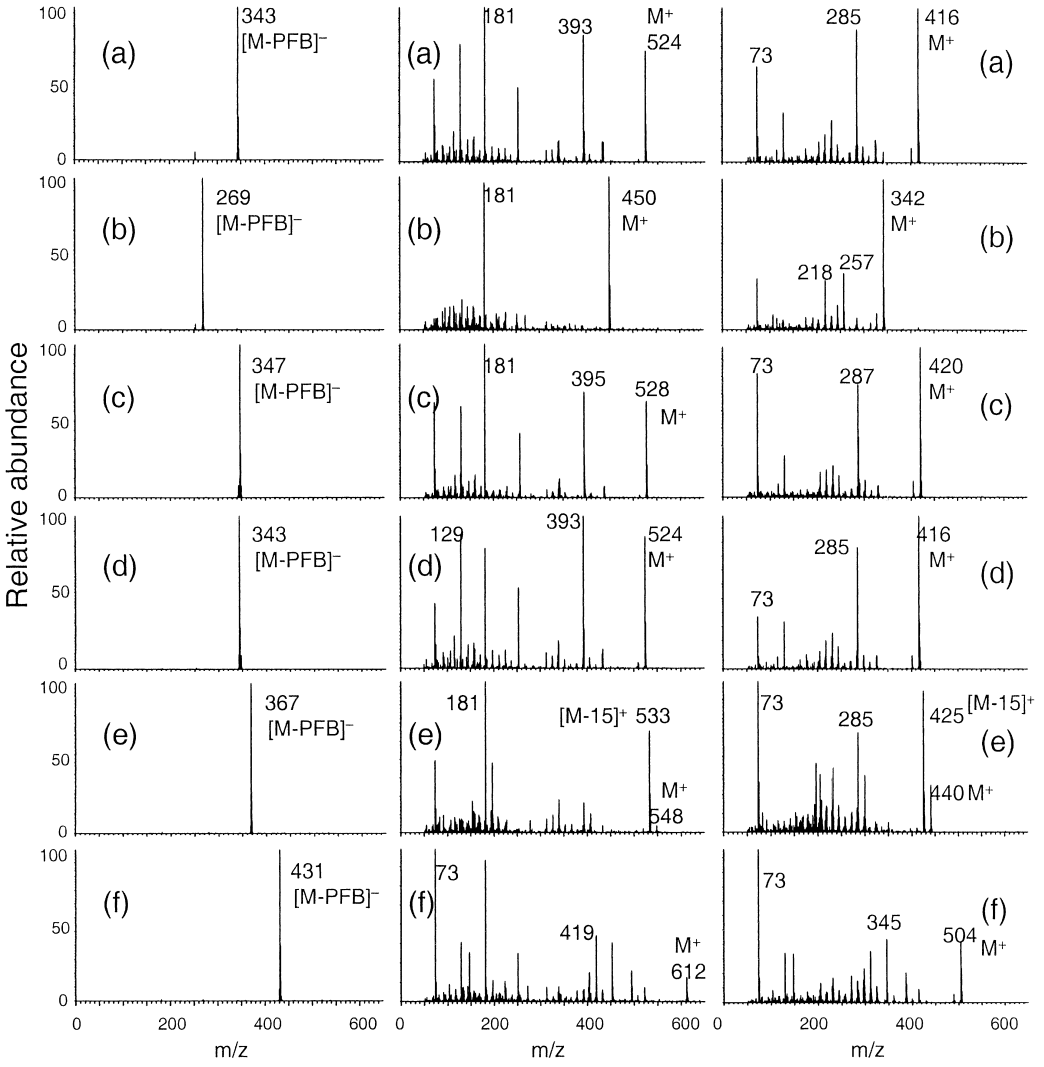

Fig. 2. NICI mass spectra of the PFB–TMS derivatives (left) and the EI mass spectra of the PFB–TMS derivatives (middle) and all the TMS derivatives (right). (a) 17α-estradiol; (b) estrone; (c) 17β-estradiol-2,4,16,16-d_4; (d) 17β-estradiol; (e): ethynyl estradiol; (f): estriol.

spectra of the PFB–TMS derivatives and EI mass spectra of the PFB–TMS derivatives and all the TMS derivatives.

3.3. Optimization of the reagent gas flow-rate and ion source temperature in the NICI mode

We have obtained the ion abundances of the PFB–TMS derivatives of the estrogens (1 ng/ml each) over the reagent gas flow-rate range between 1.0 and 3.0 ml/min (ion source temperature: 150°C) and have observed some trends. The ion abundances were obtained by SIM of the $[M-PFB]^-$ ions. The PFB–TMS derivatives of 17α-estradiol, 17β-estradiol, and estriol showed maximum ion abundances at 2.0 ml/min. The PFB–TMS derivatives of estrone and ethynylestradiol showed slightly larger ion abundances at 2.5 ml/min than those at 2.0 ml/min. We have also obtained the ion abundances of the PFB–TMS derivatives of the estrogens (1 ng/ml each) over the ion source temperature range between 150 and 270°C (reagent gas flow-rate: 2.0 ml/min) and have noted some trends. The PFB–TMS derivatives of 17α-estradiol, 17β-estradiol, and estrone showed maximum ion abundances at 240°C. The PFB–TMS derivatives of ethynylestradiol and estriol showed slightly larger ion abundances at 270°C than those at 240°C. Therefore, the reagent gas flow-rate was empirically determined as 2.0 ml/min and the ion source temperature was set at 240°C.

3.4. Determination of estrogens in SIM mode

A comparison of the detection limit levels (at a signal-to-noise ratio of 3) of the PFB–TMS derivatives by NICI-MS, the PFB–TMS derivatives by EI-MS, and all the TMS derivatives by EI-MS is shown in Table 1. The sensitivities of the PFB–TMS derivatives by NICI-MS were 8.0–130 times higher than those of the PFB–TMS derivatives by EI-MS. The sensitivities of the PFB–TMS derivatives by NICI-MS were 12–25 times higher than those of all the TMS derivatives by EI-MS. Fig. 3 shows the SIM chromatograms of the PFB–TMS derivatives (100 pg/ml each) by NICI-MS, the PFB–TMS derivatives (1 ng/ml each) by EI-MS, and all the TMS derivatives (1 ng/ml each) by EI-MS. The linearity and reproducibility of the NICI method were tested and the results are listed in Table 2. The calibration curves for all the estrogens as PFB–TMS derivatives were linear at 10, 20, 50, 100, 500, 1000, 5000, and 10000 pg/ml with correlation coefficients between 0.9993 and 0.9998. The reproducibility, expressed as a relative standard deviation (RSD) ($n=6$), for peak areas of all the estrogens as PFB–TMS derivatives were between 2.5 and 6.5% at 50 pg/ml and between 1.9 and 4.3% at 200 pg/ml.

3.5. Application to river water

3.5.1. Interference of river water

Standards (17α-estradiol and estriol, 40 pg; estrone, 17β-estradiol, and ethynyl estradiol, 400 pg: 0.2 or 2 ng/l as concentration in river water) were added to 200 ml of a river water sample, and then the river water was treated by the SPE method shown in the Experimental section. The extract spiked with the standards and the non-spiked extract were derivatized as shown in the Experimental section, and then analyzed by GC–NICI-MS. Es-

Table 1
Comparison of detection limits (at $S/N=3$) of PFB–TMS derivatives and TMS derivatives

Compounds	PFB–TMS derivatives (pg/ml)		TMS derivatives (pg/ml) EI-SIM
	NICI-SIM	EI-SIM	
17α-Estradiol	5.6	150	110
Estrone	12.5	100	220
17β-Estradiol	7.3	200	100
Ethynyl estradiol	7.5	150	190
Estriol	13.6	1760	160

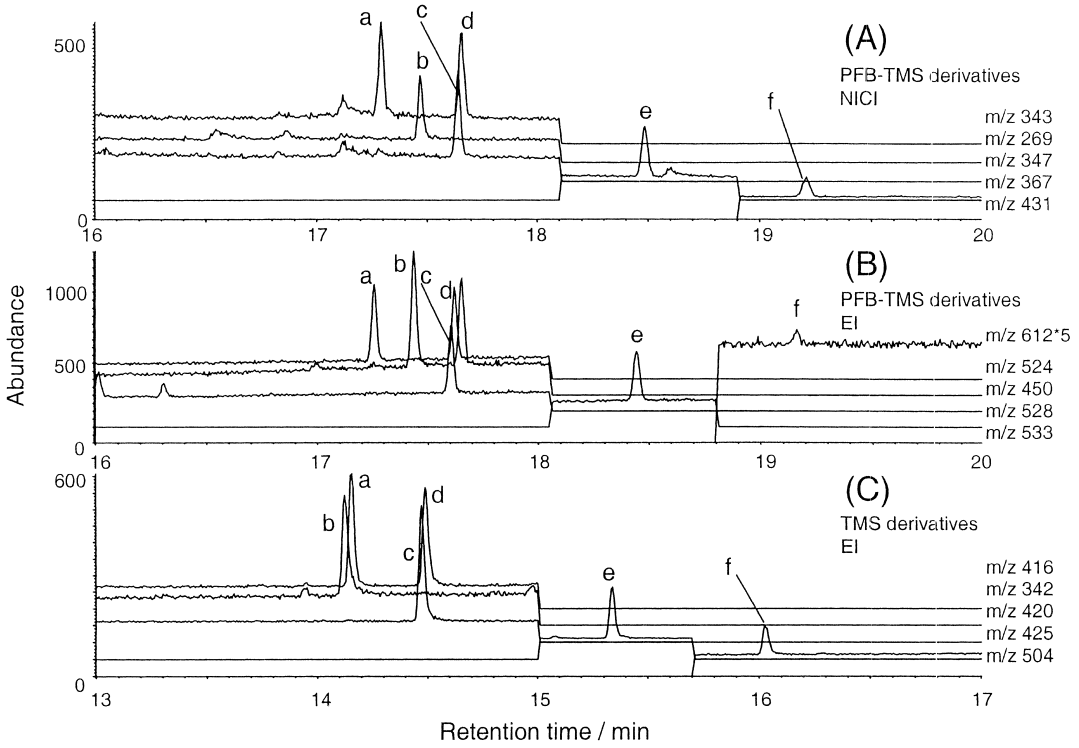

Fig. 3. NICI-SIM chromatograms of (A) the PFB–TMS derivatives of the estrogen standard mixture (100 pg/ml each) and the EI-SIM chromatograms of (B) the PFB–TMS derivatives of the estrogen standard mixture (1 ng/ml each) and (C) all the TMS derivatives of the estrogen standard mixture (1 ng/ml each). (a) 17α-estradiol; (b) estrone; (c) 17β-estradiol-2, 4, 16, 16-d_4; (d) 17β-estradiol; (e) ethynyl estradiol; (f) estriol.

trone, 17β-estradiol, and estriol were also detected in the non-spiked sample (estrone: 6.2 ng/l, 17β-estradiol: 0.7 ng/l, estriol: 0.2 ng/l). All the estrogens could be determined without interference by the river matrix. Fig. 4 shows NICI-SIM chromatograms of the PFB–TMS derivatives of the estrogens extracted from the river water spiked with the standards and from the non-spiked river water.

3.5.2. Recovery

The standards (400 pg: 2 ng/l as the concentrations in the river water) were added to 200 ml of a river water sample, and then the spiked sample was treated by the SPE method. Subsequently, the extract was analyzed by GC–NICI-MS after the derivatization. The recovery and reproducibility were tested and the results are listed in Table 3. Good recovery

Table 2
Correlation coefficients of calibration curves and reproducibilities ($n=6$)

Compounds	Monitoring ion (m/z)	Correlation[a] coefficient	% RSD at 50 pg/ml	% RSD at 200 pg/ml
17α-Estradiol	343	0.9995	5.5	4.3
Estrone	269	0.9998	4.1	2.0
17β-Estradiol	343	0.9996	6.5	3.9
Ethynyl estradiol	367	0.9993	2.5	1.9
Estriol	431	0.9997	4.9	4.3

[a] Concentration range: 10 pg/ml–10 ng/ml.

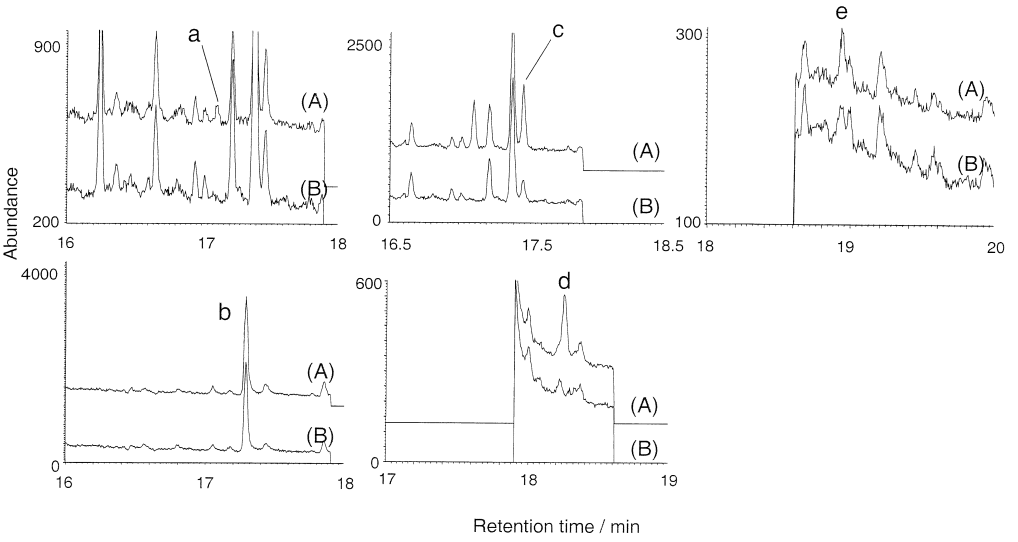

Fig. 4. NICI-SIM chromatograms of the PFB–TMS derivatives of the estrogens extracted from (A) the river water spiked with the standards, and from (B) the non-spiked river water. (a) 17α-estradiol; (b) estrone; (c) 17β-estradiol; (d) ethynyl estradiol; (e) estriol.

Table 3
Recovery (%) of estrogens from river water and reproducibility ($n=5$), and methodical detection limits[a]

Compounds	Spiked amount (pg)	Recovery (% RSD)	Methodical detection limits (ng/l)
17α-Estradiol	400	85.8 (6.2)	0.15
Estrone	400	126.5 (9.8)	0.28
17β-Estradiol	400	89.8 (7.4)	0.20
Ethynyl estradiol	400	87.3 (7.1)	0.25
Estriol	400	111.8 (13.0)	0.10

[a] River water: 200 ml.

was obtained for all the estrogens between 85.8 and 126.5% at 2 ng/l. Practical reproducibility was obtained for all the target compounds with RSD values ($n=5$) between 6.2 and 13.0% at 2 ng/l for the peak areas. The methodical detection limits are listed in Table 3, and ranged from 0.10 to 0.28 ng/l.

4. Conclusions

A sensitive GC–NICI-MS method for the determination of estrogens in river water has been developed. A significant advantage of the method is that NICI-MS provides high sensitivity for the PFB–TMS derivatives of the estrogens. Therefore, the NICI-MS allows the detection of the 0.10–0.28 ng/l level of estrogens in river water by the SPE (100-fold concentration) without a clean-up procedure. The method also provides a wide range of linearity, satisfactory recovery, and good reproducibility.

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