

Novel approaches to the analysis of steroid estrogens in river sediments

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Abstract A wide range of estrogenic contaminants has been detected in the aquatic environment. Among these, natural and synthetic steroid estrogens, typically present in municipal sewage-treatment plant (STP) effluents, are the most potent. In this study a new GC–MS method has been developed for direct analysis of five major steroid estrogens (estrone, 17β -estradiol, 17α -ethinylestradiol, dienestrol, and diethylstilbestrol) in river sediments. Four GC–MS systems used for analysis of underivatized analytes in purified extracts were compared. Relatively low detection limits (1.5 – 5 ng g⁻¹ dried sediment) and good repeatability of GC splitless injection (RSD 1–2%) were achieved by use of a system combining low-pressure gas chromatography with a single-quadrupole mass analyzer (LP-GC–MS). Use of orthogonal gas chromatography (GC×GC) hyphenated with high-speed time-of-flight mass spectrometry (HSTOF-MS) enabled not only significantly better resolution of target analytes, and their unequivocal identification, but also further improvement (decrease) of their detection limits. In addition to these outcomes, use of this unique GC×GC–TOF-MS system enabled identification of several other non-target chemicals, including pharmaceutical steroids, present in purified sediment extracts.

Keywords Pharmaceuticals · Steroid estrogens · Contaminants · Sediment · GC/MS · Non-target screening

Introduction

Discussion of the risks associated with the occurrence of a variety of groups of persistent organic pollutants (polychlorinated biphenyls and related organochlorine compounds, brominated flame retardants, modern pesticides and their metabolites, etc.) in the aquatic environment started to focus on their endocrine-disrupting properties also in early nineteen-nineties. In the last decade natural and synthetic steroid hormones have also attracted attention because of their high estrogenic potency. In common with other endocrine-disrupting chemicals (EDCs) they may induce a variety of disorders, for example abnormalities in reproduction of wildlife, in particular feminization of a male fish [1].

Not only natural 17β -estradiol and its major metabolites (estrone and estriol) but also other synthetic estrogens enter the aquatic ecosystem via effluents from municipal sewage-treatment plants (STPs). For example, 17α -ethinylestradiol, one of the most common chemicals in the latter group, is widely used as a human contraceptive and/or as the active ingredient of preparations aimed at management of menopausal and postmenopausal syndrome. This substance is also used in physiological replacement therapy in deficiency conditions, and in treatment of prostate and/or breast cancer [2]. In addition to compounds in legal use, several strictly banned estrogens (European union directive 96/22/CE [3]), for example dienestrol and diethylstilbestrol, which used to be used as growth promoters during fattening of cattle, have recently been reported to occur in river sediments [2, 3]. The log K_{OW} values of these environmental estrogenic compounds fall in the range 2.5 (estriol) to 5.3 (dienestrol), i.e. they are medium polar to relatively non-polar substances. Extensive adsorption of more hydrophobic substances on to sediment and/or sludge particles has been documented [6]. Because of the potential

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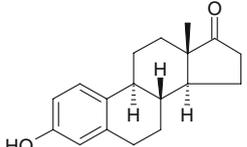
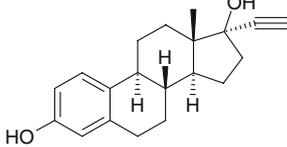
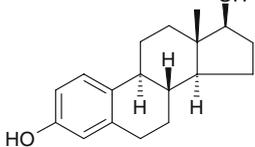
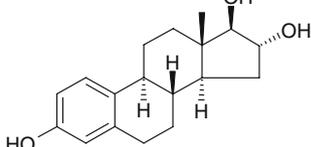
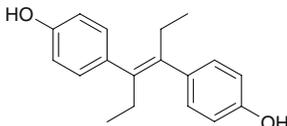
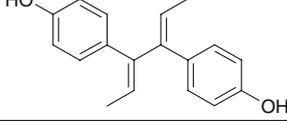
simultaneous sorption of many other aquatic pollutants on these matrices, analysis of estrogens in such complex mixtures is obviously a difficult task; not surprisingly, few papers describing (sometimes very complicated) sample-handling techniques have been published [6–8]. Typically, in the first step, estrogens are extracted from sediment or sludge by use of polar or medium polarity organic solvents or their mixtures (e.g. methanol, acetone, hexane–acetone, diethyl ether–hexane). Ultrasound-assisted extraction or pressurized-liquid extraction (PLE) can be used to enhance extraction efficiency and the speed of the process [4, 7]. In the next step, solid-phase extraction (SPE) employing a wide range of adsorbents is commonly applied for purification of the crude extract. In this step, gas chromatography coupled with mass spectrometry (GC–MS) is often used for analysis of estrogens in environmental samples. To increase spectral resolution, and hence improve detection selectivity, tandem mass spectrometry (GC–MS–MS) can be used for their determination in such complex

matrices as sludge from domestic STPs [7]. To improve analyte volatility and peak shape, derivatization, e.g. silylation [6, 7, 9] or acylation [10], can also be performed before GC analysis. In the recent years, liquid chromatography–tandem mass spectrometry (LC–MS–MS) has been widely used for direct analysis of estrogens and other steroid hormones in different environmental matrices [11–13]. Slightly worse detection limits (LOQs in the range 1–5 ng g⁻¹, supposing LOD=LOQ/3) have been obtained by use of LC–MS-based methods compared with those achievable by GC–MS (LODs typically 10⁻¹ ng g⁻¹) [7, 8].

In this study several alternative GC-based approaches to direct multiresidue analysis of underivatized steroid estrogens (estrone, 17 β -estradiol, 17 α -ethinylestradiol, dienes-trol and diethylstilbestrol; Table 1) in river sediments have been investigated and critically assessed. Four GC–MS systems were used:

1. conventional GC coupled to a single-quadrupole MS analyzer;

Table 1 Characteristics of estrogenic steroids studied [14, 15]

Target estrogen	Abbreviation	CAS number	Molecular weight	log <i>K</i> _{OW}	Chemical structure
Estrone	E1	57-16-7	270.37	3.13	
17 α -Ethinylestradiol	EE2	57-63-6	296.41	3.67	
17 β -Estradiol	E2	50-28-2	272.39	4.01	
Estriol	E3	50-27-1	288.39	2.45	
Diethylstilbestrol	DES	56-53-1	268.35	5.07	
Dienestrol	DIE	84-17-3	266.34	5.32	

2. low-pressure GC (LP-GC) coupled to a single-quadrupole MS analyzer;
3. one dimensional (conventional) GC coupled to a high-speed time-of-flight (HSTOF) mass analyzer; and
4. orthogonal GC×GC coupled to HSTOF-MS.

The potential of GC×GC–TOF-MS for non-target screening of estrogenic pollutants in purified extract has also been explored.

Experimental

Chemicals

Pure standards of estrone, 17 β -estradiol hemihydrate, estriol, 17 α -ethinylestradiol, dienestrol, and diethylstilbestrol (purity 99.5, 98.9, 99.7, 99.4, 98.0, and 99.5%, respectively) were purchased from Riedel–de Haen (Germany), 20,21-¹³C₂-ethinylestradiol (purity 99%) was purchased from Cambridge Isotope Laboratories (CIL, UK), and 16,16,17-D₃-17 β -Estradiol (purity 98%) was supplied by Sigma–Aldrich (Germany).

All solvents were of analytical grade. Ethyl acetate for pesticide-residue analysis and dichloromethane for GC residue analysis were both from Scharlau (Barcelona, Spain), hexane for gas chromatography and methanol for liquid chromatography, both LiChrosolv, were from Merck (Germany), and acetonitrile for HPLC was Chromasolv from Sigma–Aldrich. Acetone, redistilled before use, was purchased from Penta (Czech Republic). Deionized water was prepared by use of a MilliQ system (Millipore, USA). Technical gases used for GC analysis were helium 6.0 and nitrogen 5.0 (both from Siad, Czech Republic). Sodium sulfate, anhydrous (Penta), used for sample desiccation, was heated to 600 °C for 7 h and, after cooling, stored in a dark vessel in a desiccator before use. Aqueous ammonia (25%, w/w) was also supplied by Penta.

Stock solutions of the estrogens, concentration 1000 $\mu\text{g mL}^{-1}$ were prepared in acetonitrile and stored at –18 °C. Working solutions (for GC analysis), which were prepared by a serial dilution of stock solutions with ethyl acetate, were stored at –18 °C for a maximum of six months. Stock solutions of 20,21-¹³C₂-ethinylestradiol (surrogate standard) and 16,16,17-D₃-17 β -estradiol (syringe standard), concentrations 1 $\mu\text{g mL}^{-1}$ and 100 ng mL^{–1} respectively, were each prepared in ethyl acetate.

Samples

Sediment samples were collected in autumn 2005 by the Hydrobiological Research Institute in Vodany (Czech

Republic) at several locations on the Vltava (Moldau) river close to Prague. Sediments from both relatively clean (Podol, upstream from Prague) and contaminated (Troja, downstream from exit of Prague STP) locations were used for method development. Total organic carbon (TOC) content was 3.4 and 7.2%, respectively.

Methods

Extraction and clean-up

Before extraction the samples were dried at 30 °C (thermostatically controlled) for ca 16 h (spiking experiments showed there were no analyte losses). Six extraction mixtures, hexane–dichloromethane, hexane–ethyl acetate, hexane–acetone, methanol–acetone (all 1:1, v/v), ethyl acetate–methanol (4:1, v/v), and dichloromethane, were tested in our study. Each sample of dried sediment (3 g) was, before further processing, spiked with 50 ng 20,21-¹³C₂-ethinylestradiol (50 μL stock solution in ethyl acetate) used as a surrogate standard, then extracted with 50 mL of the respective solvent mixture for 30 min. Ultrasonication was used to enhance extraction efficiency. The suspension was then passed through a sodium sulfate layer and the crude extract obtained was evaporated just to dryness by rotary vacuum evaporation (the last drop of solvent was removed by means of a gentle nitrogen stream. The residue was dissolved in 10 mL 10% (v/v) acetonitrile–water and quantitatively transferred on to a preconditioned SPE cartridge. The SPE systems tested in our experiments are listed in Table 2. The SPE eluate was evaporated nearly to dryness, the last drop was removed by means of a gentle nitrogen stream, and the sample was dissolved in 500 μL ethyl acetate containing 16,16,17-D₃-17 β -estradiol, as a syringe standard, before GC analysis.

GC separation and MS detection

Four GC–MS systems, characterized as described below, were used in our study for analysis of purified sediment extracts. The first (**System A**) was used in all experiments performed during optimization of sample preparation; the other three were used only for analysis of samples prepared by use of the optimized procedure.

System A: conventional GC–single-quadrupole mass analyzer

An Agilent Technologies 6890N gas chromatograph equipped with a 5975 Inert XL mass-selective detector, operated in EI mode, and a 30 m×0.25 mm i.d., 0.15 μm film thickness DB-17MS column (J&W Scientific, USA) was used for quantification of estrogens. The injection port

Table 2 Overview of SPE systems tested for optimization of clean-up

Code	SPE cartridge	Pre-conditioning	Washing	Elution
I	ENVI-Carb, 250 mg (Supelco, USA)	5 mL acetonitrile 5 mL acetonitrile–water (10:90, v/v)	2 mL water	10 mL acetonitrile
II	ENVI-Carb, 250 mg (Supelco)	5 mL methanol 5 mL acetonitrile–water (10:90, v/v)	2 mL water	10 mL methanol
III	Supelclean LC-18, 500 mg (Supelco)	5 mL acetonitrile 5 mL acetonitrile–water (10:90, v/v)	2 mL water	10 mL acetonitrile
IV	Supelclean LC-18, 500 mg (Supelco)	5 mL methanol 5 mL acetonitrile–water (10:90, v/v)	2 mL water	10 mL methanol
V	Oasis HLB, 60 mg (Waters, USA)	5 mL acetonitrile 5 mL acetonitrile–water (10:90, v/v)	2 mL water	10 mL acetonitrile
VI	Oasis HLB, 60 mg (Waters)	5 mL methanol 5 mL acetonitrile–water (10:90, v/v)	2 mL water	10 mL methanol
VII	Oasis HLB, 60 mg (Waters)	5 mL acetonitrile 5 mL acetonitrile–1% CH ₃ COOH mixture (95:5, v/v) 5 mL acetonitrile–water (10:90, v/v)	5 mL water	10 mL acetonitrile–1% acetic acid mixture (95:5, v/v)
VIII	Oasis HLB, 60 mg (Waters)	5 mL acetonitrile 5 mL acetonitrile–1% NH ₄ OH mixture (95:5, v/v) 5 mL acetonitrile–water (10:90, v/v)	5 mL water	10 mL acetonitrile–1% ammonium hydroxide mixture (95:5, v/v)

temperature was 250 °C. The oven temperature was held at 100 °C for 1 min then programmed at 50° min⁻¹ to 280 °C which was held for 10 min. The carrier gas (helium) flow rate was held constant at 1 mL min⁻¹. Pulse splitless injections (1 µL) were made by use of an Agilent Technologies 7683 autosampler with a splitless period of 1 min and a 30 psi pulse (1 psi=6894.76 Pa) held for 1 min. The interface temperature was 280 °C. The MS detector was operated in selected ion monitoring (SIM) mode; *m/z* values used for quantification and/or identification of the target analytes are summarized in Table 3. The total analysis time was 14.6 min.

System B: low-pressure GC - single quadrupole mass analyzer

The experiments were conducted with a 9 m×0.53 mm i.d. (mega-bore), 0.5 µm film thickness RTX-5 Sil MS

analytical capillary column (Restek, USA) connected to a 5 m×0.18 mm i.d. uncoated restriction column (Agilent Technologies, USA) at the inlet end. A stainless steel union in which the restriction column fit inside the mega-bore column was used as a true zero-dead-volume connection. The GC–MS instrument was the same as in System A. The injection port temperature was 250 °C. The oven temperature was held at 100 °C for 1 min then programmed at 60° min⁻¹ to 170 °C and then at 45° min⁻¹ to 290 °C which was held for 3 min. He was used as carrier gas at a constant inlet pressure of 20 psi. The injection volume was 5 µL, the splitless period 1 min at 50 psi, and the interface temperature 280 °C. The MS detector was operated in SIM mode. Ions used for quantification of the target analytes were the same as for System A (Table 3). The total analysis time was 7.6 min.

Table 3 GC–MS analysis of selected estrogenic steroids (system A and system B)

Analyte	Retention time (min)		SIM	
	System A (GC–Q–MS)	System B (LP–GC–Q–MS)	Quantification ion (<i>m/z</i>)	Confirmation ions (<i>m/z</i>)
Diethylstilbestrol	6.2	7.2	268	145, 239
Dienestrol	6.5	7.3	266	237, 251
17β-Estradiol	8.5	8.5	272	160, 213
Estrone	8.6	8.4	270	146, 185
17α-Ethinylestradiol	9.0	8.7	296	160, 213
20,21- ¹³ C ₂ -Ethinylestradiol ^a	9.0	4.4	298	160, 213
16,16,17-D ₃ -17β-Estradiol ^b	8.5	4.3	275	160, 213

^a Surrogate standard

^b Syringe standard

System C: one dimensional (conventional) GC–MS–high-speed time-of-flight (HSTOF) mass analyzer

A Pegasus 4D instrument (Leco, USA) consisting of an Agilent 6890N gas chromatograph equipped with split/splitless injector and a Leco Pegasus III high-speed time-of-flight mass spectrometer was used for the experiments. A 30 m×0.25 mm i.d., 0.25 μm film thickness, DB-5MS column (J&W Scientific) was used for separation of sample components. The injection port temperature was 250 °C. The oven temperature was held at 80 °C with 2 min then programmed at 55° min⁻¹ to 320 °C which was held for 3.5 min. The carrier gas (helium) flow was held constant at 2 mL min⁻¹. Pulse splitless injections (1 μL) were made by means of an MPS2 autosampler (Gerstel, Germany); the splitless period was 2 min and the pulse 110 psi. The interface temperature was 280 °C. The MS acquisition rate was 10 Hz, the mass range 35–550 amu, the ion-source temperature 220 °C, and the detector potential –1800 V. The total analysis time was 10 min.

System D: orthogonal GC×GC–MS - high-speed time-of-flight (HSTOF) mass analyzer

The system was similar to **System C** except that a dual-stage jet modulator and secondary oven were mounted inside the GC oven. Resistively heated air was used as a medium for hot jets; cold jets were supplied by gaseous

nitrogen, secondary-cooled by liquid nitrogen. Orthogonal (GC×GC) separation was achieved by use of a 30 m×0.25 mm i.d., 0.25 μm film-thickness DB-5MS column (J&W Scientific) in the first dimension and a 2.2 m×0.1 mm i.d., 0.1 μm film thickness BPX-50 column (SGE International, Australia) in the second dimension. The injection port temperature was 250 °C. The primary oven temperature was maintained at 80 °C for 2 min then programmed at 55° min⁻¹ to 320 °C which was held for 3.5 min. The secondary oven was programmed with a 10-degree temperature offset. Modulation time was 7 s (2 s hot pulse) and temperature offset was 20°. Carrier gas (helium) flow rate was held constant at 2 mL min⁻¹. Pulse splitless injections (1 μL) were made with splitless period of 2 min and a pulse of 110 psi. The interface temperature was 280 °C. The MS acquisition rate was 150 Hz, the mass range 35–550 amu, the ion-source temperature 220 °C, and the detector potential –1800 V.

Validation and quality control

The method was validated in accordance with Commission Decision 2002/657/EC, Part 3, Annex I [16]. Our approach is summarized in Scheme 1. In the first phase, two sets of dry blank sediments, each consisting of six samples, were spiked with the analyte mixture at two concentrations (15 and 150 ng g⁻¹). After incubation for 2 h the samples were processed as described above (hexane–acetone extraction,

Scheme 1 Summary of the validation study (R_{EX} , recovery in the extraction step; R_{SPE} , recovery in the clean up step; R_{WM} , recovery of the whole method)

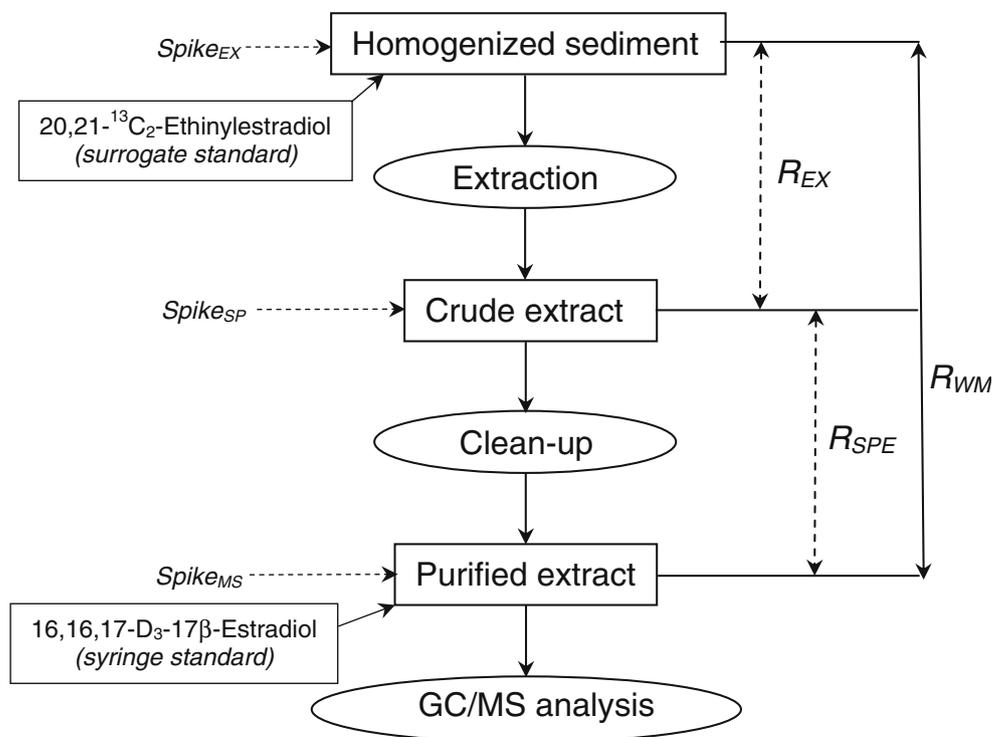


Table 4 Performance of SPE systems tested for optimization of clean-up (crude extract spiked at 150 ng g⁻¹, the equivalent of 3 g dried sediment was loaded on the cartridge)

Code ^a	Recovery R_{SPE} (%)				
	Estrone	Estradiol	Ethinylestradiol	Dienestrol	Diethylstilbestrol
I	Not eluted from the cartridge under these experimental conditions ^b				
II	Not eluted from the cartridge under these experimental conditions ^b				
III	59	13	46	54	64
IV	74	59	68	76	68
V	84	86	87	104	100
VI	75	48	72	75	67
VII	92	88	94	96	78
VIII	113	102	116	96	98

^a Experimental details are given in Table 2

^b Elution might require back-flush

SPE clean-up with system VIII, GC–MS system A for quantification). As shown in Scheme 1, extraction recovery R_{EX} was estimated by measurement of total recovery R_{WM} and recovery in SPE R_{SPE} (crude extract spiked with mixture of standards). Precision of measurements was expressed as a relative standard deviation. For quantification, a multilevel calibration plot was used (at least five points for each analyte in the range 5–1,000 ng mL⁻¹). To prepare a matrix-matched standard 3 g dried sediment (real sample with no detectable native content of target compounds) were processed as described above, the residue after evaporation of the SPE fraction was dissolved in 0.5 mL ethyl acetate standard solution containing syringe standard and 100 ng mL⁻¹ of each analyte, equivalent to dried sediment contaminated at 15 ng g⁻¹. In Scheme 1 matrix-matched standard preparation is indicated Spike_{MS}. Limits of quantification (LOQ) were set as concentrations three times higher than the limit of detection (LOD). LOD was estimated as three times the signal-to-noise (S/N) ratio.

Results and discussion

The first phase of our project was implementation of an analytical procedure for examination of river sediments for the presence of both natural and synthetic steroid estrogens.

Table 5 Recovery R_{WM} and repeatability for the whole method (System A employed for quantification, spiking level 15 ng g⁻¹ and 150 ng g⁻¹ dried sediment, each analyte)

Analyte	Recovery, R_{WM} (%)		Repeatability (RSD, %)	
	15 ng g ⁻¹	150 ng g ⁻¹	15 ng g ⁻¹	150 ng g ⁻¹
Estrone	83	120	6	5
Estradiol	127	133	7	7
Ethinylestradiol	96	116	5	4
Dienestrol	60	58	17	12
Diethylstilbestrol	75	91	5	5

LC–MS was our first choice of method. In preliminary experiments with the objective of optimization of detection limits (LODs) a standard mixture containing six target estrogens (estrone, 17 β -estradiol, estriol, 17 α -ethinylestradiol, dienestrol, and diethylstilbestrol) was separated on a reversed-phase C₈ column (250 mm \times 4 mm, 5 μ m LiChro-CART RP-8, Merck, Germany) with an acetonitrile–water gradient as mobile phase. Two types of tandem mass spectrometric detector—one an ion trap (Finnigan LCQ Deca, USA) and the other a triple quadrupole (Waters Quattro Premier XE, USA)—were used for analyte detection. Rather surprisingly, we failed to achieve detection limits below 1 ng injected for E2 and EE2 (under conditions used for sample preparation, this amount corresponds to a sediment contamination level of 10 ng g⁻¹ dried sediment); for other standard mixture components the values were only slightly lower. Considering the high probability of analyte signal suppression (hence a further increase of the LODs) because of the adverse matrix effects commonly encountered in analysis of real samples, we decided to explore potential of an alternative separation and detection strategy. Gas chromatography (GC) coupled to mass spectrometry (MS) was chosen as a technique that may enable monitoring of even trace levels of estrogenic steroids in river sediments. In contrast with the conventional approach based on volatilization by silylation or acylation before GC determination [6, 7, 17], we attempted to analyze target compounds directly, without derivatization. The results of thorough optimization of sample preparation and selection of optimum GC–MS conditions for identification and quantification are summarized below.

Sample preparation

As already mentioned in the Introduction, the K_{OW} values (polarity—Table 1) of the estrogens studied in this work vary over a relatively wide range and, for this reason, finding an optimum strategy enabling their simultaneous isolation is not an easy task. Six extraction solvent mixtures (Experimental section) were tested to identify that resulting in the highest recoveries for most of the analytes. On the

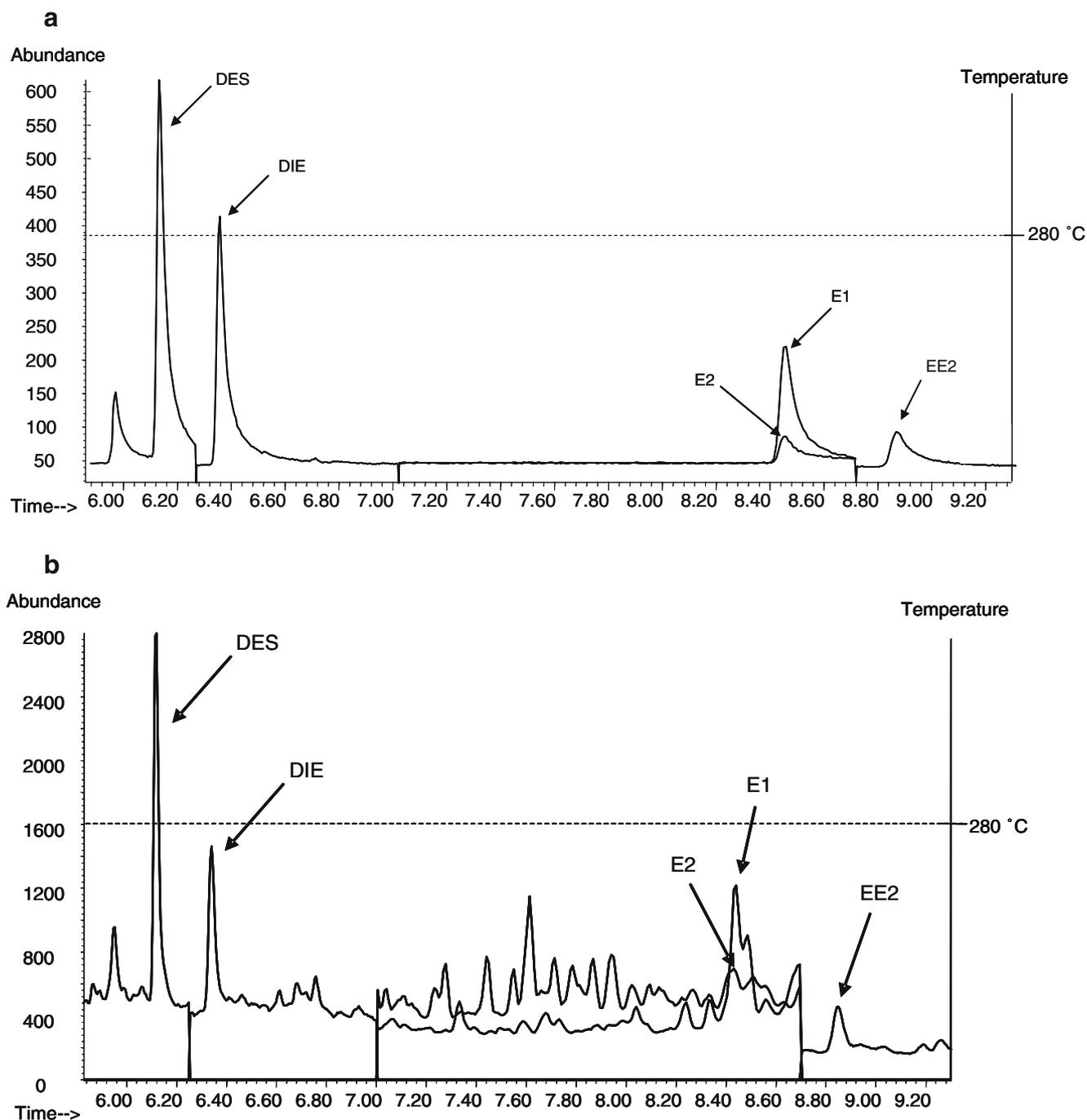


Fig. 1 a GC–MS (System A) chromatogram (SIM) obtained from a standard mixture (100 ng mL^{-1} in ethyl acetate); extracted quantification ions (m/z) for DES, DIE, E2, E1 and EE2 were 268, 266, 272, 270 and 296, respectively. **b** GC–MS (System A) chromatogram

(SIM) obtained from matrix-matched standard mixture (100 ng mL^{-1} each analyte, corresponding to 15 ng g^{-1} dried sediment); the extracted ions are the same as for Fig. 1a

basis of the data generated, hexane–acetone was chosen for follow-up experiments, because this led to the best results under the optimized clean-up conditions (recoveries, R_{EX} , were 111, 92, 96, 86, and 97% for E1, E2, EE2, DIE, and DES, respectively).

For clean-up we started with gel-permeation chromatography (GPC) on Bio-Beads S-X3 and 1:1 (v/v) cyclohexane–

ethyl acetate as mobile phase. Unfortunately, this approach, widely used for purification of a variety of environmental extracts failed to separate the target analytes from other material co-extracted from the matrix. The elution volumes of the steroid estrogens investigated in our study were rather lower than those of other common persistent organic pollutants (POPs), for example PCBs, OCPs, PAHs and/or

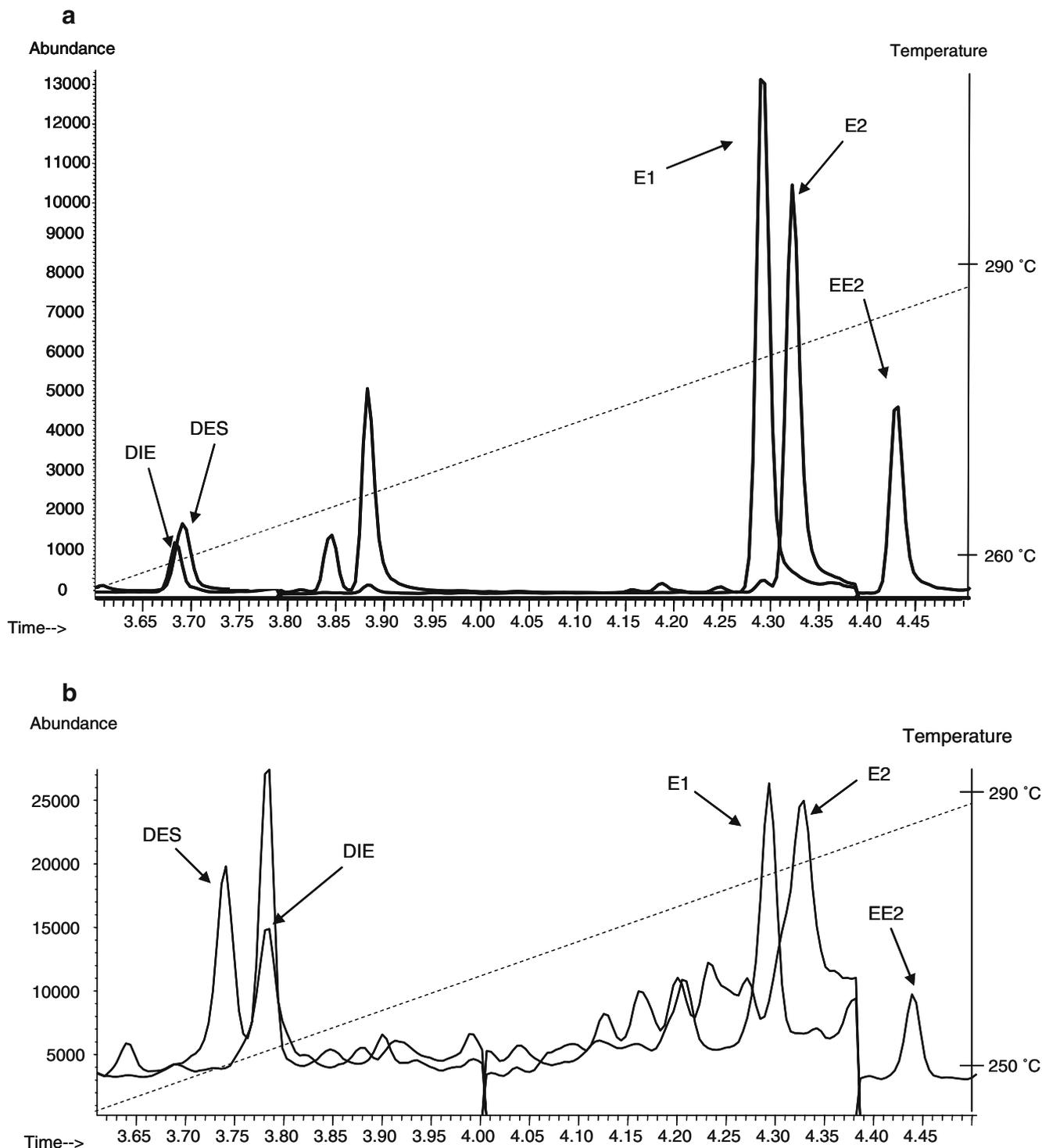


Fig. 2 **a** LP-GC-MS (System B) chromatogram (SIM) obtained from standard mixture (100 ng mL^{-1} in ethyl acetate); extracted ions are the same as for Fig. 1a. **b** LP-GC-MS (System B) chromatogram (SIM)

obtained from matrix-matched standard mixture (100 ng mL^{-1} of each analyte, corresponding to 15 ng g^{-1} dried sediment); extracted ions are the same as for Fig. 1a

BFRs, often present in river sediments, hence there was some overlap with the elution bands of impurities. As an alternative, several solid-phase extraction (SPE) systems were examined in the search for an optimum clean-up strategy; the results are summarized in Table 4.

Among the systems tested, SPE clean-up with Oasis HLB cartridges in combination with alkaline acetonitrile as elution solvent (system VIII) was identified as the best option not only with regard to mean recoveries but also because of good repeatability (not exceeding 4%). Results

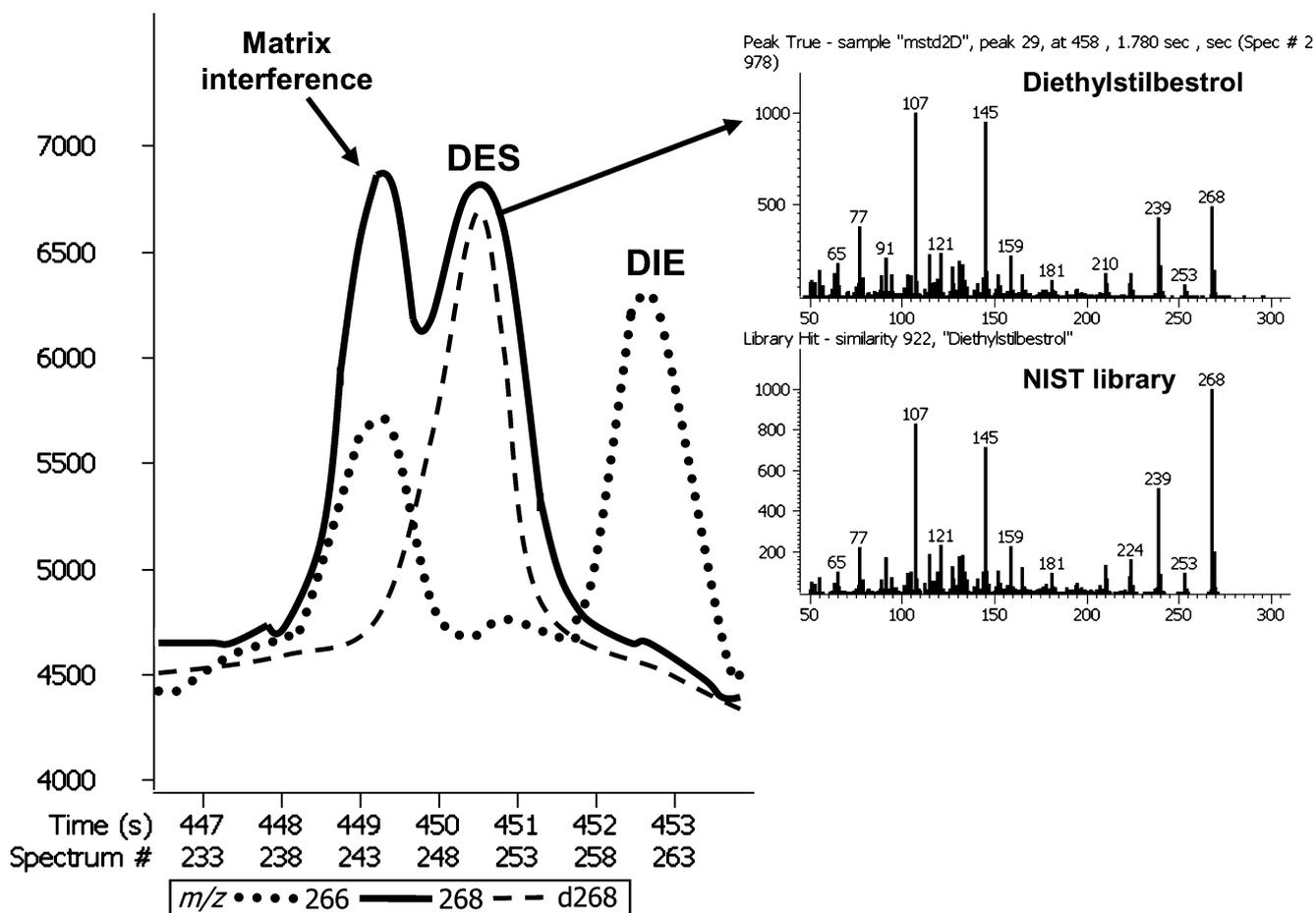


Fig. 3 GC-TOF-MS (**System C**) chromatogram obtained from diethylstilbestrol (DES) and dienestrol (DIE) in matrix-matched standard (100 ng mL⁻¹ each analyte, corresponding to 15 ng g⁻¹

dried sediment). The *dashed line* is the deconvoluted trace for the ion *m/z* 268 and shows the exact position of the DES peak

from validation of the method using conventional GC-MS **System A** (**Experimental** section) for quantification of the target analytes in spiked river sediment are shown in Table 5.

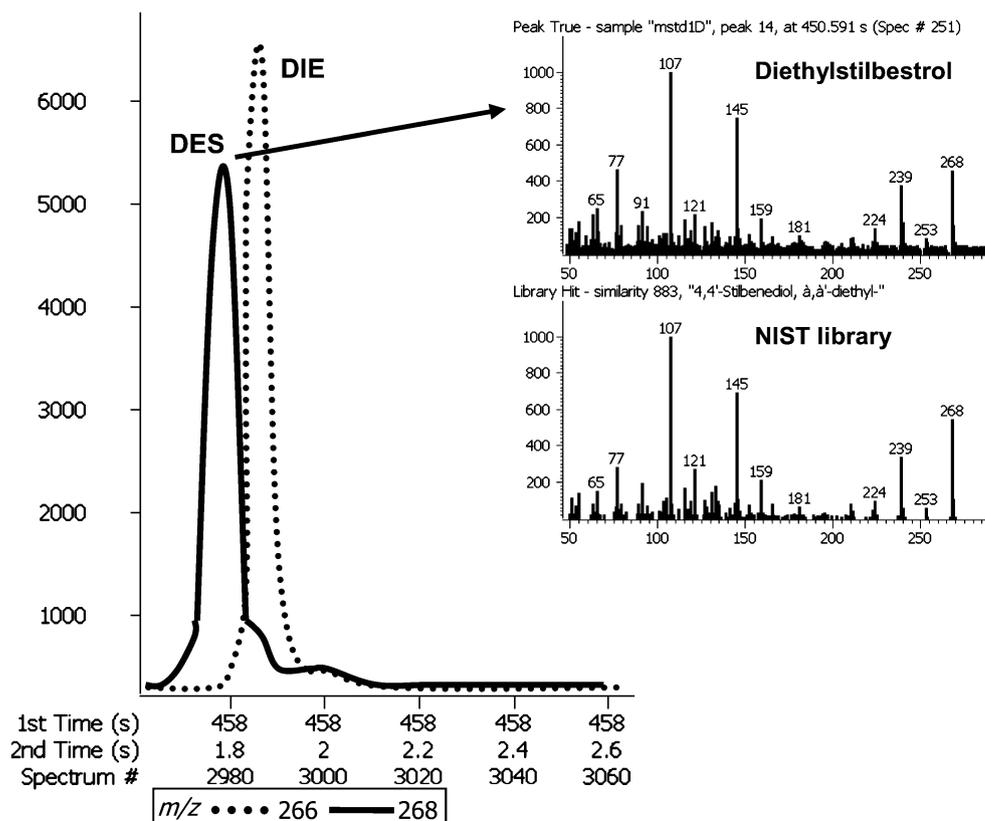
It should be noted that the main objective of this study was to document the feasibility of direct GC-MS analysis of steroid pharmaceuticals in river sediments. The performance characteristics achieved depend on all the steps of the procedure and can, obviously, be further improved by using a system other than conventional **System A** for validation.

Separation and detection systems

As shown in Fig. 1a, pronounced tailing of the target analyte peaks was observed when their standard mixture was injected into GC **system A** (estriol, which eluted as a very broad band at 13 min, is not shown here). These problems (peak asymmetry) were obviously because of interactions of polar groups in the target steroids (for example three OH groups capable of hydrogen bonding in estriol) with active

sites in the GC system that are (unavoidably) present both in the injection port and in the GC column. On the basis of the chromatogram shown in Fig. 1a, derivatization of analytes may seem an inevitable option. As is apparent from Fig. 1b, however, derivatization was not needed when analyzing real samples containing matrix co-extracts (poorly volatile estriol was not included in further studies). Significant improvement of the shapes and intensities (heights) of the DES, DIE and EE2 peaks was achieved. A complex phenomenon responsible for these effects, so-called matrix-induced chromatographic response enhancement, has been described in detail in one of our previous papers [18]. Briefly, masking of active sites by co-injected matrix components prevents the occurrence of the adsorption phenomena causing not only analyte peak tailing but also reduction of the number of molecules reaching the GC detector. Under these conditions, only use of matrix-matched standards and/or application of isotope dilution techniques can prevent overestimation of the amounts of semi-polar analytes that may occur when external calibration based on standards in pure solvent is used (Fig. 1a).

Fig. 4 GC×GC–TOF-MS (System D) chromatogram (System D) chromatogram obtained from diethylstilbestrol (DES) and dienestrol (DIE) in matrix-matched standard (100 ng mL⁻¹ each analyte, corresponding to 15 ng g⁻¹ dried sediment)



For the most polar, later-eluting analytes (E1, E2, and EE2) LODs were, unfortunately, relatively high, not only because of the low specificity of quantification ions used (for E1 and E2 detection) but also because of band-broadening in time at an isothermal plateau (280 °C) where they were eluted. Because neither high-resolution nor tandem (MS–MS) mass spectrometric detectors were available for this project, we attempted to achieve better detectability of these analytes by use of low-pressure (LP) GC–MS (System B). This technique [19, 20], employing an uncoated restriction capillary column in front of the separation column (an arrangement that enables use of normal splitless injection) connected to a mega-bore capillary column operated under vacuum conditions, because of its direct connection to the MS source, enables not only injection of a larger volume of sample (5 µL in this work), because of the higher column capacity, but also more rapid elution of analytes under the programmed gradient at lower temperatures (Fig. 2a). Significant reduction of peak tailing was achieved by use of system b even for standards in pure solvent (compare Fig. 2a with Fig. 1a); analyte signal heights were, however, still substantially less than when matrix-matched standards were used (Fig. 2b).

It should be noted that use of a low-resolution single quadrupole mass analyzer in GC–MS Systems A and B did not enable sufficiently selective detection of the late eluting analytes, despite the relatively high m/z values of their

quantification ions. Because of fairly intense chemical noise, the detection limits achieved with both conventional and low-pressure GC–MS systems were rather high. In these circumstances, either improved chromatographic resolution or better spectral separation of the sample components were conceivable options for solving these problems. In the next set of experiments, System C, consisting of a conventional GC column coupled to a high-speed TOF-MS detector, was used to investigate the latter possibility. This unique detection technique enables acquisition of full mass spectral information even at low analyte levels (the duty cycle of the TOF analyzer is typically as high as 20–30%, in contrast with 0.1–1% for scanning instruments). Because of a rapid acquisition rate and the absence of spectral skew, deconvolution and unbiased identification of target analytes is possible even when they overlap with compounds yielding similar m/z ions. Figure 3 shows an example of deconvoluted DES in matrix-matched standard (concentration 15 ng g⁻¹ dried sediment). As shown here, DES can be identified from NIST library search. It should, however, be noted that LODs achieved by GC–TOF-MS were still relatively high for some compounds, worse even than for routine system A. Significant improvement of LODs was achieved by use of system D in which a TOF-MS detector was coupled with orthogonal, comprehensive GC (GC×GC). Separation of matrix interferences, because of the high peak capacity of this chromatographic system, with peak compression by

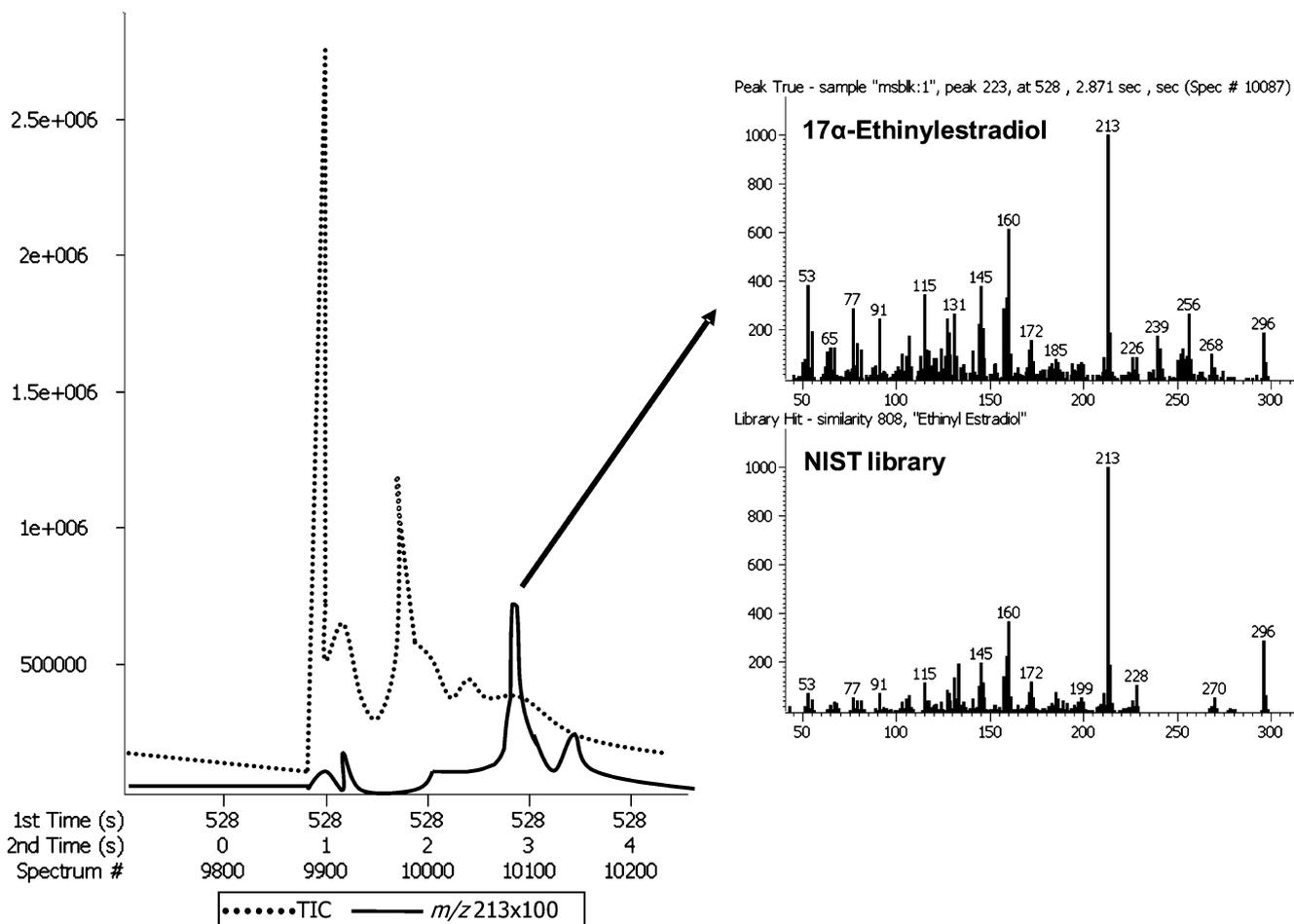


Fig. 5 GCxGC–TOF-MS (System D) detection/quantification of native 17α-ethinylestradiol in river sediment; locality Troja

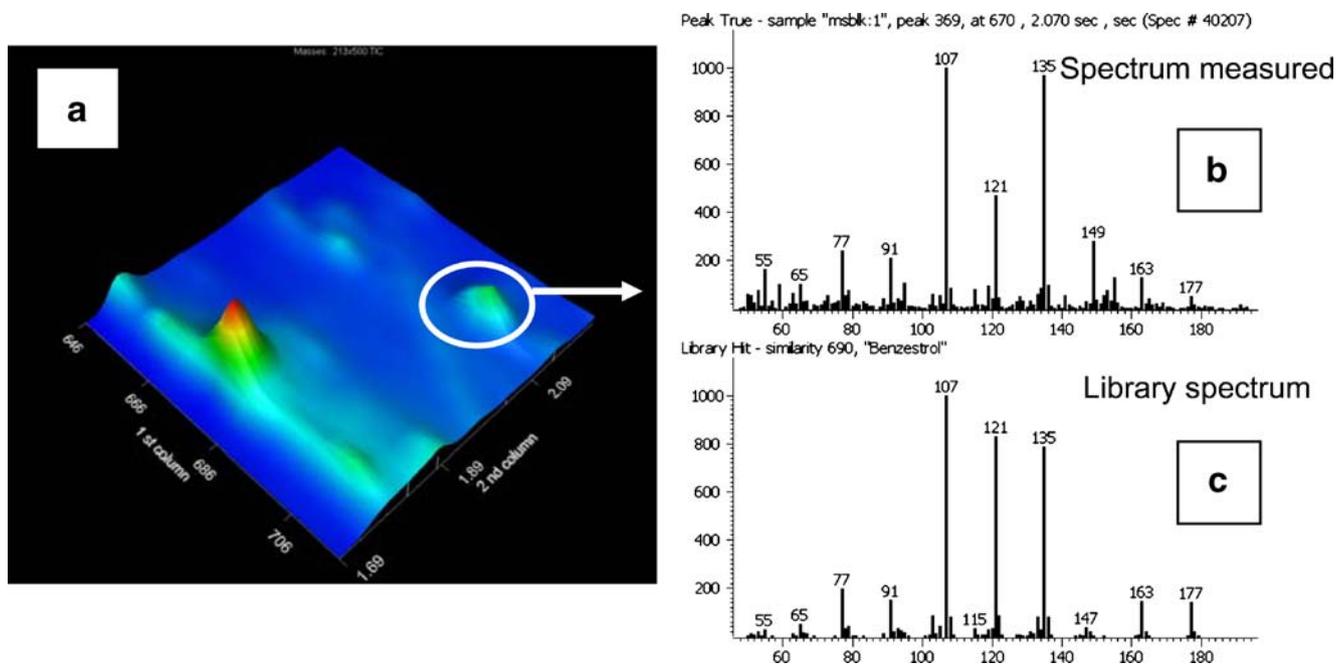


Fig. 6 Identification of benzestrol in a sample of river sediment by use of the non-target screening option: (a) zoomed part of GCxGC contour plot (benzestrol is marked by white circle); (b) spectrum obtained from native benzestrol; (c) NIST library spectrum of benzestrol

Table 6 The most abundant compounds identified by non-target search within the data generated by GC×GC–TOF-MS (system D) analysis of purified sediment extract

Matrix coextract	CAS	Spectral match
α-Sitosterol	83-46-5	944
Heptacosane	593-49-7	947
Heptadecane	629-78-7	928
Diocetyl adipate	123-79-5	932
Diisobutyl phthalate	84-69-5	985
Oleic acid, ethyl ester	111-62-6	892
Diethylhexyl phthalate	84-75-3	920
Hexadecanoic acid, butyl ester	111-06-8	863

cryo-focusing occurring during their transfer on to the second-dimension column, resulted in the best, i.e. lowest, LODs among the systems tested. The distinct improvement of *S/N* ratios is well illustrated for DES and DIE in Fig. 4. The values increased from 46 and 67 in System C to 259 and 403, respectively, in system D. The capability of GC×GC–TOF-MS to detect even trace levels of steroid pharmaceuticals in real sediment samples is well illustrated by Fig. 5. The concentration of native ethinylestradiol was, however, below the limit of quantification (LOQ), which we estimate to be approx. 0.5 ng g⁻¹. It is worth mentioning that comparison of the ethinylestradiol spectrum with that in the NIST library reveals some mass peaks (239, 256 ...) in the compound spectrum which are not present in the NIST spectrum. Despite this, the identification quality match, 808, was still relatively high for such a trace level of the analyte (many ions were used for identification).

With regard to characterization of compounds contained in sample extracts, the high potential of GC×GC–TOF-MS was also revealed by use of the non-target screening option enabled by the Leco software ChromaTOF. Several other hormonal pharmaceuticals, both androgenic and estrogenic compounds, were found in the purified sample; one example, identification of the estrogenic compound benzestrol is shown in Fig. 6. It should be noted that the clean-up procedure used in this study was optimized for recovery of five target estrogens. Under these conditions many other compounds, including non-target

contaminants, might have been completely or partly removed during processing of the crude extract. Despite these potential losses, the presence of a wide spectrum of trace contaminants, mainly polycyclic aromatic hydrocarbons (PAHs), which are typically associated with river sediments, was revealed. Similarly, the chlorinated persistent pollutants dieldrin and *p,p'*-DDE were discovered by searching for “unknowns”. Altogether, 632 peaks were detected; some of the most abundant (semi)volatile sample components identified in the purified extract are listed in Table 6 (only compounds with a spectral match exceeding the value 860 are shown).

Selected performance characteristics of the four GC systems for analysis of matrix-matched standards (i.e. samples containing matrix components not removed by the purification procedure) are compared in Table 7. The response of all the detectors tested was a linear function of concentration up to at least 150 ng g⁻¹ dried sediment (the upper concentration of the matrix-matched calibration standard). The correlation coefficients within this range were not lower than 0.9999. Relative repeatability of measurements was best in LP-GC–MS (system B), which was shown to be the most robust in this respect.

Conclusions

To the best of our knowledge this is the very first study demonstrating the possibility of direct GC–MS analysis of trace levels of underivatized steroid estrogens in river sediments. If a low-resolution single-quadrupole mass analyzer, only, is available for analysis of sediment extracts, LP-GC–MS is a suitable option for routine analysis, not only because of rapid separation of sample components at low temperatures but also because of its potentially lower detection limits compared with conventional systems. For further reduction of detection limits, reduction of the risk of false positives, and/or non-target screening of steroids and other contaminants present in sample extracts, comprehensive GC×GC–TOF-MS is an excellent tool, both because the enormous chromatographic resolution reduces interferences and because

Table 7 Limits of detection (LODs), in ng g⁻¹ dried sediment, and repeatability of six splitless injections, expressed as RSD, % (at the level 15 ng g⁻¹ dried sediment) for the GC–MS systems tested

Analyte	System A GC–Q-MS (SIM mode)		System B LP-GC–Q-MS (SIM mode)		System C GC–TOF-MS		System D GC×GC–TOF-MS	
	LOD	RSD	LOD	RSD	LOD	RSD	LOD	RSD
Estrone	5	9	1.5	2	10	8	2.5	9
Estradiol	5	12	5	2	12	16	2.5	18
Ethinylestradiol	5	7	1.5	2	1.5	4	0.4	6
Dienestrol	1.5	7	5	1	7	6	1.5	4
Diethylstilbestrol	1.5	6	1.5	1	7	6	1.5	3

of the availability of full spectral information searchable in an MS library at even low analyte levels. Irrespective of the GC system used, matrix-matched standards and/or labeled internal standards must be used to compensate for the matrix-induced chromatographic response enhancement to which relatively polar analytes, for example the examined estrogenic pharmaceuticals, are susceptible. Only this calibration strategy provides accurate results.

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