Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1017 (2003) 167–185
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

# Quantitation of estrogens in ground water and swine lagoon samples using solid-phase extraction, pentafluorobenzyl/trimethylsilyl derivatizations and gas chromatography–negative ion chemical ionization tandem mass spectrometry

Dennis D. Fine [a,*], G. Peter Breidenbach [b], Treaver L. Price [c], Stephen R. Hutchins [d]

[a] *Shaw Environmental and Infrastructure, P.O. Box 1198, Ada, OK 74821-1198, USA*
[b] *ManTech Environmental Research Services Corporation, P.O. Box 1198, Ada, OK 74821-1198, USA*
[c] *MCAAP, 1 C-Tree Road, McAlester, OK 74501, USA*
[d] *Ground Water and Ecosystems Restoration Division, US Environmental Protection Agency National Risk Management Research Laboratory, P.O. Box 1198, Ada, OK 74821-1198, USA*

Received 17 April 2003; received in revised form 15 July 2003; accepted 12 August 2003

**Abstract**

A method was developed for the confirmed identification and quantitation of 17β-estradiol, estrone, 17α-ethynylestradiol and 16α-hydroxy-17β-estradiol (estriol) in ground water and swine lagoon samples. Centrifuged and filtered samples were extracted using solid-phase extraction (SPE), and extracts were derivatized using pentafluorobenzyl bromide (PFBBR) and *N*-trimethylsilylimidazole (TMSI). Analysis was done using negative ion chemical ionization (NICI) gas chromatography–mass spectrometry–mass spectrometry (GC–MS–MS). Deuterated analogs of each of the estrogens were used as isotope dilution standards (IDS) and were added to the samples before extraction. A limit of quantitation of 1 ng/l in ground water was obtained using 500 ml of ground water sample, 1.0 ml of extract volume and the lowest calibration standard of 0.5 pg/μl. For a 25 ml swine lagoon sample, the limit of quantitation was 40 ng/l. The average recovery of the four estrogens spiked into 500 ml of distilled water and ground water samples ($n = 16$) at 2 ng/l was 103% (S.D. 14%). For 25 ml of swine lagoon samples spiked at 500, 1000 and 10,000 ng/l, the average recovery for the four estrogens was 103% (S.D. 15%). The method detection limits (MDLs) of the four estrogens spiked at 2 ng/l in a 500 ml of ground water sample ranged from 0.2 to 0.6 ng/l. In swine lagoon samples from three different types of swine operations, estrone was found at levels up to 25,000 ng/l, followed by estriol and estradiol up to levels at 10,000 and 3000 ng/l, respectively. It was found that pretreatment of swine lagoon samples with formaldehyde was necessary to prevent conversion of estradiol to estrone.


*Keywords:* Water analysis; Environmental analysis; Derivatization, GC; Isotopic dilution; Estrogens; Endocrine disruptors

* Corresponding author. Tel.: +1-580-436-8669; fax: +1-580-436-8635.
*E-mail address:* fine.dennis@epa.gov (D.D. Fine).

0021-9673/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2003.08.021

## 1. Introduction

The occurrence of endocrine-disrupting chemicals (EDCs) in surface water is becoming of increasing concern worldwide, and has led to a growing awareness that animal, and perhaps human, health and function in ecosystems might become negatively impacted by continued release of EDCs into the environment. Some of the most potent EDCs include both natural and synthetic estrogens, which are either produced endogenously by animals or used as pharmaceutical products in both human and veterinary medicine [1]. The natural estrogens include 17β-estradiol (estradiol), 16α-hydroxy-17β-estradiol (estriol), and estrone, which are generally more biodegradable than synthetic estrogens such as 17α-ethynylestradiol (ethynylestradiol). Although these compounds can be degraded biologically, they have been detected in sewage treatment effluents and receiving surface waters at nanogram per liter levels [2–5]. These concentrations are significant, because research has shown that male fish exposed to low nanogram per liter levels of these estrogens, either intermittently or continuously, will exhibit estrogenic responses, such as vitellogenin production [6,7].

In addition to sewage treatment plants, concentrated animal feeding operations (CAFOs) also constitute a source for release of natural estrogens into the environment, although there have been few studies on the impact of these operations. Theoretically, this impact could be significant, given that livestock can produce estrogens in large quantities and that animal wastes are generally untreated [8]. Disposal by land application is generally used to take advantage of the nutrient values of animal waste, and is used for waste litter from poultry operations, for solid waste from cattle and dairy operations, and for liquid waste and runoff from cattle, dairy, and swine operations [9]. The problem is exacerbated in that the economic trend is toward fewer operations with larger numbers of animals, resulting in larger quantities of animal wastes that must be handled within smaller areas [10]. Release of estrogens from CAFOs has been documented for runoff from poultry farms [11] and for karst ground water impacted by poultry and cattle operations [12]. There is virtually no information on the release of estrogens from swine operations and the potential for impact on ground water. This is an area of concern, because these operations are becoming more prevalent in the central/southwestern regions of the US, where sparse rainfall, relative lack of surface water streams, and a general increased depth to ground water promotes land application of CAFO waste.

To assess potential impacts on ground water and help develop more comprehensive land management strategies, sensitive and reliable analytical techniques are required. Many methods are available for the determination of estrogens in ground water [13,14], but due to the complexity of swine lagoon water, only those methods used for sewage influent/effluent or river water were considered. Although sensitive liquid chromatography–mass spectrometry–mass spectrometry (LC–MS–MS) methods using electrospray ionization (ESI) [4] and atmospheric pressure chemical ionization (APCI) [15] are available, both these methods are susceptible to response loss due to ion suppression caused by matrix effects present in complex samples [16–18]. When ion suppression is present, additional preparative steps including silica gel clean up or utilization of LC–LC methods may be required. For liquid chromatography methods, derivatization is not usually employed but several publications report increased sensitivity when estrogens are derivatized [19]. With gas chromatographic methods, derivatization of the phenolic and hydroxy groups of the estrogen is done to achieve additional selectivity and to improve gas chromatographic peak elution. The highest sensitivity for the GC–MS methods is obtained when negative ion chemical ionization (NICI) is used to determine estrogens having pentafluorobenzyl (PFB) [20–22], pentafluorobenzoyl [23,24] and other fluorine containing derivatives [25]. In all the cases where influent or river water samples were analyzed, the determinative method involved selective reaction monitoring (SRM) with MS–MS. The use of tandem mass spectrometry provides added specificity which is necessary when analyzing samples of increased matrix complexity, as found in swine lagoon water. To aid in determining whether GC–MS–MS or LC–MS–MS analysis should be done on a sample, preliminary screening of ground water samples for the presence of estrogens can be done using the enzyme-linked immunosorbent assay (ELISA). The ELISA method detection limit (MDL) for estradiol is reported to be 20 ng/l. In order to detect estradiol at a desired level of 1 ng/l, at least 100 ml of aqueous

sample should be processed using solid-phase extraction (SPE). For municipal wastewater effluents, Huang and Sedlak [26] showed that additional sample cleanup of the SPE extract using HPLC was required before analyzing the sample by ELISA. The additional clean up was required to remove interfering natural organic matter which gave false positive responses.

The aim of this study was to develop a SPE, derivatization, and GC–MS–MS method that allows determination of estrogens in swine lagoon samples and, with only minor modification of sample volumes and calibration ranges, also allows determination of estrogens in ground water. The four target estrogens included the natural estrogens estradiol, estriol, and estrone, as well as the synthetic estrogen ethynylestradiol (Fig. 1). Although natural estrogens are excreted primarily in conjugated forms, this method was developed for analysis of the free forms, because conjugated estrogens are expected to be relatively short-lived in the environment [2,4]. Ethynylestradiol was also selected because this compound is more potent than natural estrogens and, while not expected to be found in swine lagoon effluents, could be detected in ground waters which might also be impacted by human waste. The analytical method reported here is based on Nakamura et al.'s [20] derivatization of the phenolic group in the estrogen with pentafluorobenzyl bromide (PFBBR) and the hydroxy group(s) with *N*-trimethylsilylimidazole (TMSI), and subsequent GC–MS analysis using selected ion monitoring with NICI. Modifications to their method include the use of different solid-phase media for extraction, addition of a preservative for sample integrity, addition of deuterated isotope dilution standards (IDS), and the use of GC–MS–MS with selected reaction monitoring (SRM). The modified method was then used to determine estrogens in waste lagoons from three different types of swine CAFOs.

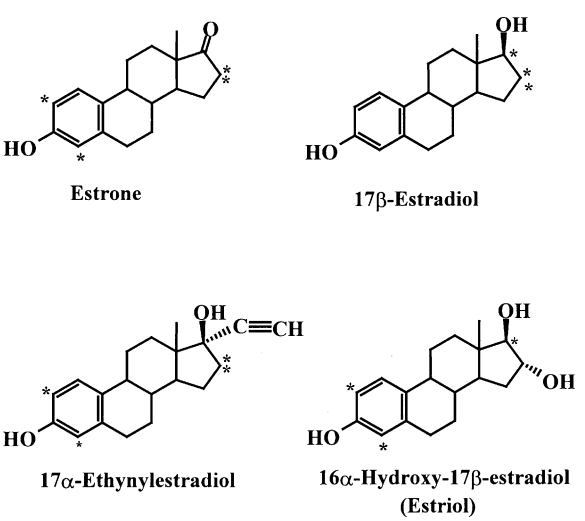


Fig. 1. Structures of estrogens with deuterium positions labeled with asterisks.

## 2. Experimental

### 2.1. Chemicals

Formaldehyde (37%), methanol (99.93%), acetone (>99.9%), methyl *tert*-butyl ether (MTBE) (>99%), hexane (99%), ammonium hydroxide (28%) and reagent water (99.5%) were obtained from Sigma–Aldrich. TMSI and Sylon CT (5% dimethyldichlorosilane in toluene) were purchased from Supelco. Anhydrous potassium carbonate (99.0%) was obtained from Fluka and was fired at 600 °C for 4 h before storage in a drying cabinet. Anhydrous sodium sulfate (analytical grade) was purchased from Mallinckrodt and was fired at 400 °C for 12 h. Table 1 provides a list of target estrogens and deuterated estrogens used as IDS. The table also provides the purity and source of each compound. The structural positions where deuteriums are located are identified in Fig. 1. Glass fiber filters, APFC (90 mm, particle retention > 1.2 μm) were purchased from Millipore. Empore glass filter aid was purchased from 3 M. For SPE, 6 ml glass cartridges containing 200 mg of Oasis HLB (hydrophilic lipophilic balance) were obtained from Waters. Estrogen standards were prepared by dissolving weighed quantities of solid standards in acetone.

### 2.2. Calibration standards

Separate calibration curves were prepared for ground water and lagoon samples. For ground water samples, the concentrations of target estrogens in the calibration standards were 0.1, 0.5, 1.0, 5.0 and 10.0 pg/μl. The IDS were present at 5.0 pg/μl. When the ground water sample volume was 500 ml and the

Table 1
Target estrogens, deuterated estrogens and surrogate

| Compound | Class | $M_r$ | Purity | Source |
|---|---|---|---|---|
| 17β-Estradiol | Target | 272.4 | 97% | Aldrich |
| Estrone | Target | 270.4 | >99% | Aldrich |
| 17α-Ethynylestradiol | Target | 296.4 | 98% | Aldrich |
| 16α-Hydroxy-17β-estradiol (estriol) | Target | 288.4 | 98% | Aldrich |
| [16,16,17-$d_3$]17β-Estradiol | IDS | 275.4 | 98 at.% D | Aldrich |
| [2,4,16,16-$d_4$]Estrone | IDS | 274.4 | 95 at.% D | Aldrich |
| [2,4,16,16-$d_4$]17α-Ethynylestradiol | IDS | 300.4 | >98 at.% D | C/D/N |
| [2,4,17-$d_3$]16α-Hydroxy-17β-estradiol | IDS | 291.4 | 98 at.% D | C/D/N |

IDS indicates isotope dilution standard.

final extract volume was 1.0 ml, extract concentrations equivalent to the lowest calibration standard, 0.1 pg/μl, would correspond to a sample concentration of 0.2 ng/l. When lagoon samples were analyzed, the range of calibration standard concentrations for lagoon sample extracts was 1, 5, 10, 50, 100, 250 and 1000 pg/μl. The concentration of the IDS in the final extract solution was 100 pg/μl. For a lagoon sample volume of 25 ml and a final extract volume of 1.0 ml, extract concentrations equivalent to the lowest calibration standard (1.0 pg/μl) corresponded to a sample concentration of 40 ng/l.

### 2.3. Sample collection, preparation and preservation

#### 2.3.1. Sample collection

Ground water and swine lagoon effluent samples were obtained from selected facilities in Oklahoma representing three types of operation: (1) nursery, (2) finisher, and (3) farrowing sow. Nursery operations typically manage weaned pigs (more than 10–15 lb; 1 lb = 0.9540 kg) and ship "feeder" pigs (40–60 lb) to growing–finishing operations. The selected nursery facility for this study utilized one uncovered anaerobic lagoon per barn complex, which in this case was designated NUR1. Finisher operations typically manage 40–60 lb pigs and "finish" these to market weights of about 225 lb. The selected finisher facility for this study utilized one uncovered anaerobic lagoon per barn complex, which in this case was designated FIN1. Farrowing sow operations typically breed pigs and ship 10–15 lb pigs to nursery operations. The selected farrowing sow facility for this study utilized a series of five anaerobic lagoons for two barn complexes, with liquid manure being first directed to two covered digester lagoons (SOW1 and SOW2), then to two uncovered primary lagoons (SOW3 and SOW4), and finally to an uncovered secondary lagoon (SOW5). Ground water and swine lagoon effluent samples were obtained directly from selected monitoring wells and swine lagoons, respectively, using either submersible pumps or PTFE bailers. Samples were transferred directly to clean 500 ml amber jars with PTFE-lined lids and shipped on ice to the laboratory, where they were stored at 4 °C prior to analysis.

#### 2.3.2. Preservation of lagoon samples

Sample splits of some lagoon samples were amended with formaldehyde [27] to test the efficacy of this preservation method. For these samples, 2.7 ml of 37% formaldehyde was added to 100 ml of lagoon sample. The sample was carefully stirred if high alkalinity was present and was not sealed until $CO_2$ evolution stopped. Formaldehyde was not added to ground water samples.

#### 2.3.3. Centrifugation and filtration of lagoon samples

Before SPE, the lagoon samples were centrifuged and filtered. If samples contained high alkalinity and were preserved with formaldehyde, care was taken not to shake the sample when opening as foaming could occur due to the release of carbon dioxide pressure. Representative samples were transferred to a Nalgene centrifuge bottle, and the sample was centrifuged for 20 min at a relative centrifugal force of 6800 × $g$ using a Sorval RC5C centrifuge. The aqueous sample was carefully transferred to a filter flask without disturbing the precipitate. The sample was filtered through 50 g of 3 M glass filter aid which was placed on top of a 90 mm Millipore APFC glass fiber filter. The filter

media and flask were rinsed with glass distilled water and with a small portion of the sample before filtering the remainder of the sample.

### 2.4. Isotope dilution standard and spiking standard addition

For ground water samples, 50 μl of the 100 pg/μl IDS in acetone was added to 500 ml of the sample. Based on a final extract volume of 1.0 ml, this resulted in an IDS concentration in the extract of 5.0 pg/μl. For ground water sample spikes, 10 μl of the 100 pg/μl estrogens was added. This results in a spike concentration in the sample of 2 ng/l and in the extract of 1.0 pg/μl.

IDS and matrix spike standards were added after the lagoon samples were centrifuged and filtered. For lagoon samples, 10 μl of the 10 ng/μl IDS was added to 25 ml of the sample. Based on a final extract volume of 1.0 ml, this resulted in an IDS concentration in the extract of 100 pg/μl. For lagoon matrix spike samples, 25 μl of the 1000 pg/μl estrogens was added to 25 ml of the sample. This results in a spike concentration in the sample of 1000 ng/l and in the extract of 25 pg/μl. In some cases, the spike concentrations were adjusted to match the range of the concentrations found in the lagoon samples.

### 2.5. Solid-phase extraction

Glassware used for SPE was fired at 600 °C for 4 h and treated with 5% dimethyldichlorosilane in toluene (Sylon CT) for 1 h. After rinsing twice with methylene chloride, the glassware was dried at 140 °C for 1 h. For the SPE method as recommended by Waters [28], 6 ml Oasis HLB cartridges were placed in a Supelco Visiprep 24-port SPE manifold. The cartridges were conditioned with 3 ml of MTBE, 3 ml of methanol and 3 ml of distilled water using vacuum applied to the manifold. After disposing of the waste solvent collected in the manifold, transfer lines were attached to the top of each cartridge and the end of the line inserted into the water sample. A needle valve below each cartridge was adjusted so that the sample flow was about 3 ml/min. After the sample passed through the cartridges, the cartridges were washed with 3 ml of 40% methanol in water followed by 3 ml of glass distilled water. The cartridge was then washed with 2 ml of 10% methanol/2% ammonium hydroxide in water. Air was drawn through the cartridge for 1 h to remove excess water. Silanized 15 ml centrifuge tubes were placed below each cartridge and sorbed compounds were eluted with 6 ml of 10% methanol in MTBE. The solvent in the centrifuge tubes was then removed with nitrogen blow down in a water bath heated at 40 °C using a Meyer N-EVAP analytical evaporator. The transfer lines were cleaned by slowly drawing 15 ml of methanol–MTBE (1:1) through each tube.

### 2.6. Derivatization

The derivatization method described here is based on the method reported by Nakamura et al. [20]. The dried extracts in the 15 ml centrifuge tubes were reconstituted with 1 ml of acetone. Then 100 μl of 10% aqueous potassium carbonate and 10 μl of neat PFBBR were added. The screw cap with PTFE septum was tightened and the tubes were heated at 60 °C for 1 h. The acetone was then removed using nitrogen blow down resulting in a residual volume of 100 μl, which is the volume of the remaining aqueous solution. Next, 0.5 ml of reagent water was added to the centrifuge tube and after shaking, 2.5 ml of hexane was added. After shaking a second time, the lower aqueous layer was removed with a fired disposable pipet and discarded. About 0.5 ml of anhydrous sodium sulfate was then added and after again shaking, a disposable pipet was used to transfer the hexane extract to a second, clean centrifuge tube. This centrifuge tube was not silanized. The pipet was inserted through the sodium sulfate and as much hexane as possible was removed. Care was taken not to transfer any solid sodium sulfate with the hexane extract. The sample was blown down again and 100 μl of TMSI and 200 μl of hexane were added. The tube was rolled so that the liquid contacted all the surfaces of the tube. After a total contact time of 30 min, 800 μl of hexane was added to bring the hexane volume to 1.0 ml. The tube was shaken again and 100 μl of reagent water was added. The hexane turned cloudy and the tube became slightly warm. After continued shaking the liquid turned clear and an orange droplet of water appeared at the bottom of the centrifuge tube. The orange color was due to imidazole, a hydrolysis product of the TMSI. The upper hexane layer

was transferred to a fired 2 ml crimp cap autosampler vial.

### 2.7. Instrumentation

#### 2.7.1. Gas chromatography

GC–MS–MS analysis was done using an Agilent 7673 autosampler, an Agilent 5890 gas chromatograph and a Finnigan TSQ-7000 mass spectrometer. A DB5-XLB capillary column (60 m × 0.25 mm i.d. with a 0.25 μm film thickness, J&W Scientific, Folsom, CA, USA) was used for the separation. The GC oven was held isothermal at 150 °C for 2 min, and then temperature programmed to 300 °C at a rate of 6 °C/min with a final hold time of 33 min. The entire GC run was 60 min long. A 10 μl Hamilton gas tight syringe with a PTFE tip plunger was used for sample injection. A volume of 3 μl of sample was injected splitless for 2 min into a deactivated double tapered splitless liner. A 20 ml/min split flow was maintained after the splitless time. A gold coated injector seal was used. The front of the capillary column was inserted 2 mm past the injection port seal. The linear velocity of air injected at 80 °C with a column back pressure of 30 psi (helium) was measured at 34 cm/s (1 psi = 6894.76 Pa). Supelco Thermogreen LB-2 septa were used in the injection port. The GC injection port and GC–MS transfer line temperature were maintained at 280 and 300 °C, respectively.

#### 2.7.2. Negative ion chemical ionization mass spectrometry

For NICI mass spectrometry, the ionizer temperature was 225 °C and the manifold temperature was 70 °C. The electron current was 300 μA and the electron energy was 200 V. Methane was used as the reagent gas for NICI. The source pressure was optimized by maximizing the intensity of the ion at *m*/*z* 633 of FC-43 (perfluorotributylamine) observed in the negative ion mode. For maximum sensitivity with methane as a chemical ionization gas, care was taken to remove all possible sources of oxygen from the instrument [29]. The helium carrier gas was scrubbed using oxygen traps and the chemical ionization gas manifold of the TSQ-7000 was removed and replaced with a Nupro “B” series sealed bellows on/off valve and crimped stainless steel capillary tubing (0.001 in. i.d.; 1 in. = 2.54 cm). The capillary tubing was crimped so that a regulated methane pressure of 18 psi provided an indicated source pressure of 6000 mTorr (1 Torr = 133.322 Pa). The removable ion volume was cleaned with aluminum oxide (600 grit), rinsed with water and methanol and then dried at 150 °C before starting each sample queue. As indicated by Skarping et al. [30], method sensitivity was improved by removing about 10 mm of polyimide coating at the end of the capillary column and inserting the capillary to within 2 mm of the center of the ion volume. Minimum heating with a micro torch was used to char the polyimide coating. The end of the capillary was wiped with a methanol soaked paper towel to remove the entire residue. The presence of the fused silica inside the ion volume did not adversely affect the automatic tuning of the instrument.

Before starting the mass calibration, 1 μl of 1,1,1-trichloroethane (TCA) was delivered into a capped 20 ml bottle. After allowing the vapor to equilibrate, 10 μl of TCA headspace was delivered into the GC injection port with the split valve turned off and the oven temperature set at 30 °C. This caused TCA to elute continuously into the ion source within the time frame required to calibrate the mass spectrometer. The NICI mass spectrum of TCA exhibits a strong negative ion at *m*/*z* 35 due to the almost complete fragmentation of the molecule to the chloride ion. Addition of this ion to the mass calibration target ion list extended the calibration range of the mass spectrometer to mass values lower than would normally be possible with the calibration gas, FC-43.

#### 2.7.3. Mass spectrometry–mass spectrometry

For MS–MS operation, argon at a pressure of 3.0 mTorr was used as the collision induced dissociation gas. Table 2 shows the parameters which were used for SRM. The precursor ion in each case is the negative phenoxy ion resulting from the loss of the pentafluorobenzyl (PFB) group. If the estrogen has hydroxyl groups then one or two trimethylsilyl (TMS) functional groups are present. The product ion for each estrogen and the optimum collision energy were determined by selecting the most intense product ion while the collision energy was varied. For enhanced method sensitivity in MS–MS, the mass resolution in Q1 was adjusted until the peak width at half height for ions in the calibration gas standard was 1.0 u.

Table 2
MS–MS parameters used for estrogens and isotope dilution standards

| Derivatized estrogen | Class | Precusor ion (u) | Product ion range (u) | Collision energy (V) | Scan time (s) |
|---|---|---|---|---|---|
| Estrone-PFB | Target | 269.2 | 144.7–145.7 | 35 | 0.25 |
| Estrone-PFB-2,4,16,16-$d_4$ | IDS | 273.2 | 146.7–147.7 | 35 | 0.25 |
| 17β-Estradiol-PFB-TMS | Target | 343.2 | 252.7–253.7 | 35 | 0.25 |
| 17β-Estradiol-PFB-TMS-16,16,17-$d_3$ | IDS | 346.2 | 254.7–255.7 | 35 | 0.25 |
| 17α-Ethynylestradiol-PFB-TMS | Target | 367.2 | 276.7–277.7 | 28 | 0.25 |
| 17α-Ethynylestradiol-PFB-TMS-2,4,16,16-$d_4$ | IDS | 371.2 | 280.7–281.7 | 28 | 0.25 |
| 16α-Hydroxy-17β-estradiol-PFB-$(TMS)_2$ | Target | 431.2 | 340.7–341.7 | 35 | 0.25 |
| 16α-Hydroxy-17β-estradiol-PFB-$(TMS)_2$-2,4,17-d | IDS | 434.2 | 342.7–343.7 | 35 | 0.25 |

IDS indicates isotope dilution standard.

## 3. Results and discussion

### 3.1. MS–MS spectra

Figs. 2–5 show typical product ion spectra of the derivatized target estrogens and deuterated estrogens which were obtained at the optimum collision energy. As expected with PFB derivatives, the molecule undergoes dissociative electron capture resulting in the loss of the PFB group and formation of a phenoxy anion. The TMS functional group, if present, is retained in the precursor ion. For the four target estrogens, the predominant collision induced fragment ions occur as a result of charge-remote fragmentations [31] through two different pathways. In the estrone-PFB product ion spectrum (Fig. 2), the ion at *m/z* 145 results from

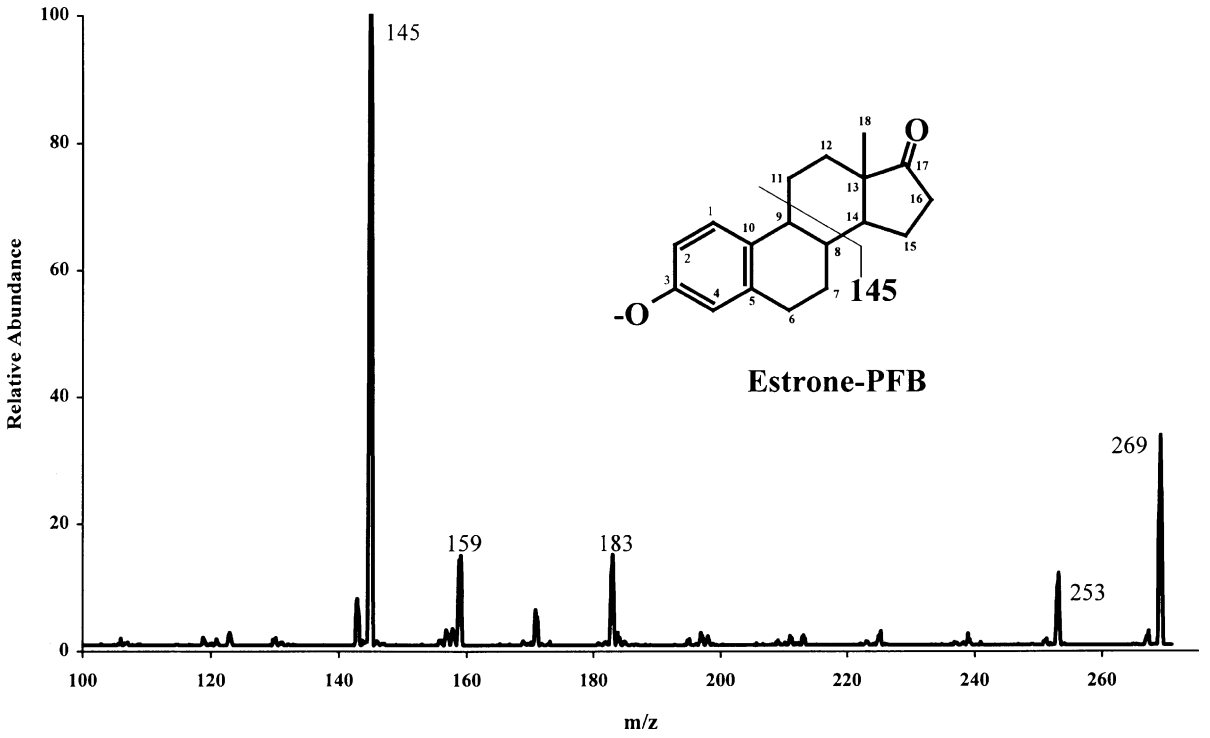


Fig. 2. Product ion spectrum of estrone-PFB.

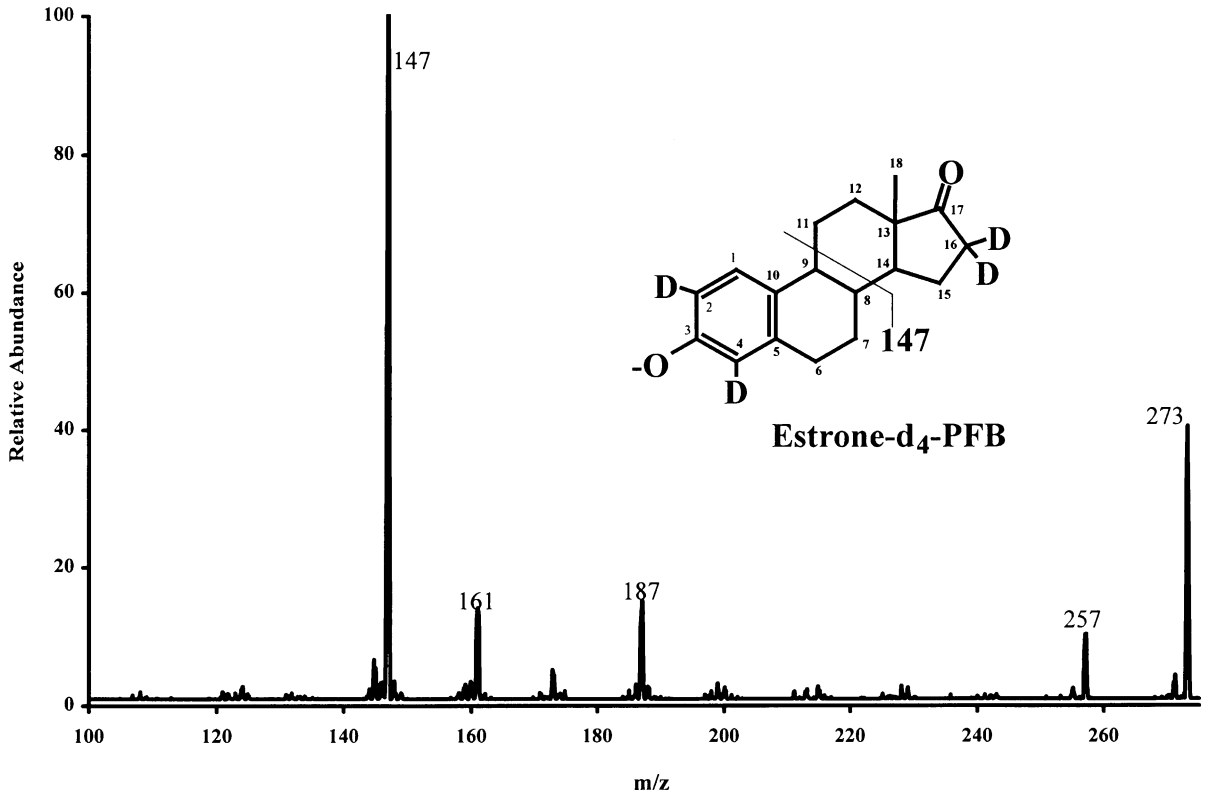


Fig. 3. Product ion spectrum of estrone-$d_4$PFB.

fragmentation of the C-ring and formation of a double bond between the 9- and 8-positions [19,21]. In the product ion spectrum of estrone-[2,4,16,16-$d_4$]PFB (Fig. 3), the deuterium atoms at the 2- and 4-positions are retained in the fragment ion, and an ion at *m*/*z* 147 results. For derivatized estradiol which has a TMS group attached to the oxygen at the 17-position, the analogous fragment loss of the C-ring is not as intense. The predominant fragment ion at *m*/*z* 253 is due to a 1,4-elimination of trimethylsilanol, $HOSi(CH_3)_3$. In the product ion spectrum of 17β-estradiol-[16,16,17-$d_3$]PFB-TMS (Fig. 5), an ion due to the loss of $^2HOSi(CH_3)_3$ is found at *m*/*z* 255. Similar fragment losses are found in the product ion spectra of ethynylestradiol-[2,4,16,16-$d_4$]PFB-TMS and estriol-[2,3,17-$d_3$]PFB-$(TMS)_2$ (spectra not shown). Comparison of the overall mass spectral response of the four estrogens showed that estrone-PFB gave greater response than the other three estrogen–PFB-TMS derivatives. Estriol-PFB-$(TMS)_2$ had the lowest response.

### 3.2. GC–MS and GC–MS–MS

Figs. 6 and 7 show SRM mass chromatograms of two lagoon samples, FIN1 and SOW5, and the same lagoon samples spiked to an extract concentration of 250 and 25 pg/μl, respectively. The mass chromatograms for each unspiked and spiked sample are overlapped within each figure. When the target compound is present in the sample, the spiked compound coelutes with the target compound. When the same spiked and unspiked extracts from lagoon sample FIN1 were run using GC–NICI–MS without collisional fragmentation, unknown peaks, as shown in Fig. 8, were found to overlap with estrone-$d_4$ and estriol. Considerable baseline fluctuation was found in the mass chromatograms for ethynylestradiol-$d_4$,

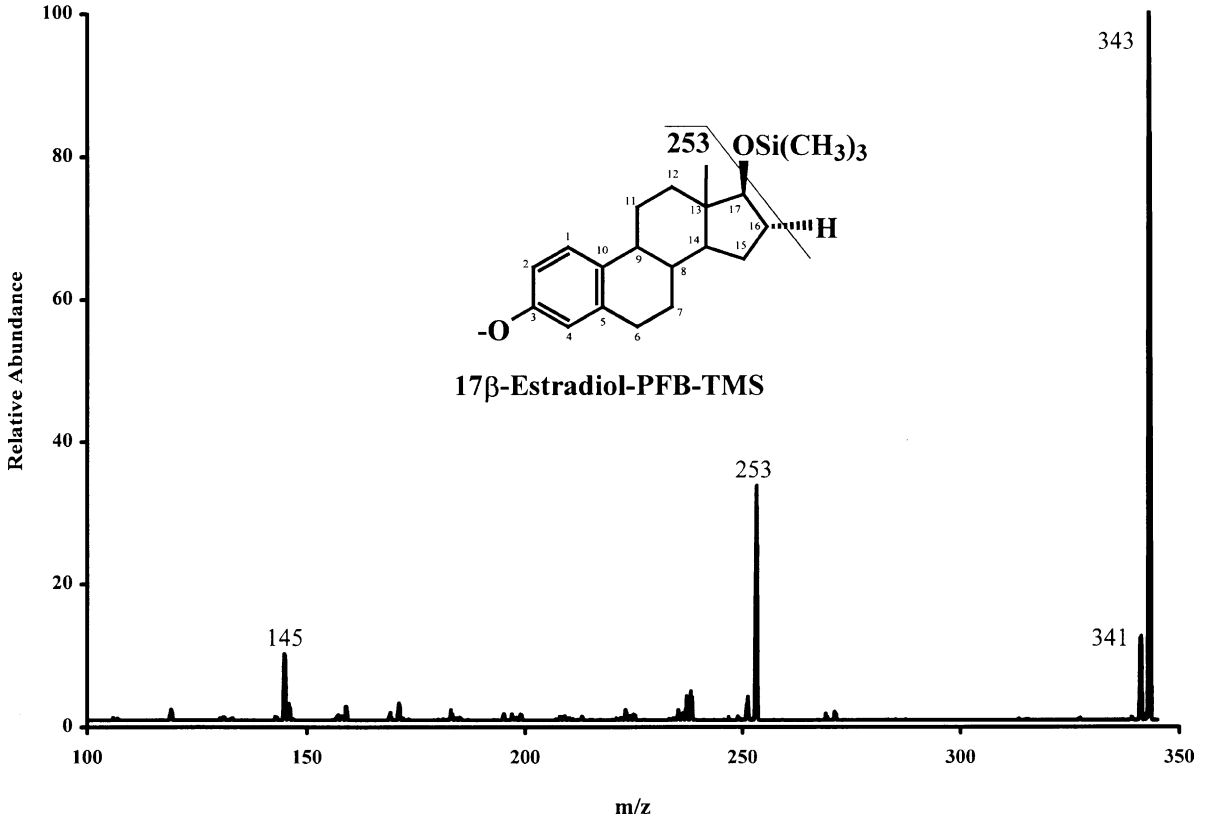


Fig. 4. Product ion spectrum of 17β-estradiol-PFB-TMS.

estriol and estriol-$d_4$. In the GC–NICI–MS mass chromatograms, the displayed ions were extracted from the full scan spectrum. Although comparison of Figs. 6 and 8 shows increased specificity is obtained using MS–MS, Fig. 7 provides an example where that specificity is not enough. When estriol was spiked into the SOW5 lagoon sample, a peak much broader than expected was found. The broader peak resulted from the spiked estriol coeluting with an unknown compound. Although increased specificity can sometimes be gained by choosing a second strong ion formed during collision, this was not possible with the derivatized estriol. Only one strong ion due to the loss of $HOSi(CH_3)_3$ was present in the product ion spectrum. Even if an alternative SRM ion set was possible it may not help if the unknown peak was a diasteriomer of estriol. Since two chiral carbons are present in estriol at C16 and C17, two pairs of stereoisomers, diasteriomers, are possible.

### 3.3. Method performance

#### 3.3.1. Method response

Changes in the overall method response were monitored by following the peak area of IDS in each sequential sample. For ground water samples and standards where the IDS were present in the extract at 5.0 pg/μl, the peak area of all the IDS decreased slightly with each run. In addition to the derivatized estrogens, dibenzophthalate which was initially added to the final extract as a instrument profile standard also experienced the same response decrease. When the ion volume was cleaned, the response of the estrogens and dibenzophthalate immediately increased. This decrease in peak area was more significant for low level standards where the total amount of individual deuterated estrogens injected on column was 15 pg compared to 300 pg for lagoon samples. It was found that the loss in response was not due to contamination

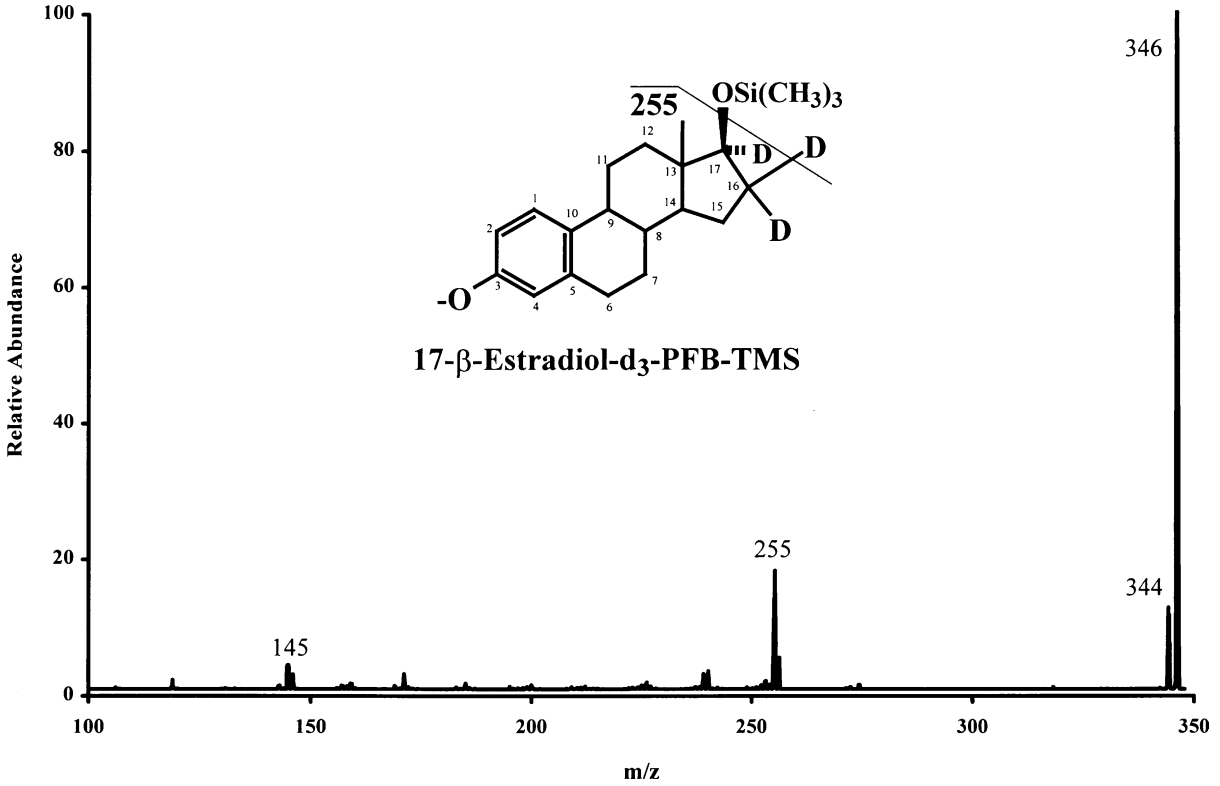


Fig. 5. Product ion spectrum of 17β-estradiol-$d_3$PFB-TMS.

of the ion volume by cumulative material injected in the sample extracts. The peak area decrease was the same after the filament was left on for 6 h within 3 runs as compared to 16 runs with the filament on for the same period of time. The cause of the sensitivity loss may be due to reaction of rhenium oxides from the filament with the chemical ionization reagent gas (methane) and formation of a residual material that coats the surface of the ion volume [29]. The effect of the contamination was minimized by adding an oxygen scrubber to the helium carrier gas line and making the chemical ionization gas transfer lines leak tight. When a clean ion volume was installed, the background spectrum contained ions at *m/z* 17, 35 and 235 corresponding to $OH^-$, $Cl^-$ and $ReO_3^-$. The ratio of the intensities of these ions was 7:1:1. The $ReO_3^-$ ion disappeared as the filament warmed up. The $OH^-$ remained the 100% ion but this ion also faded into the base line noise as the filament and ion volume were thermally conditioned. Also the length of time the filament was on was minimized to 10 min during the run cycle. The sensitivity of the method was also increased by moving the capillary column to within 3 mm of the center of the ion volume [30]. Initially, the end of the capillary was about 13 mm from the center of the ion volume and eluting compounds had to travel through a transfer hole in the ion heater block to reach the ion volume. With the end of the capillary column moved inside the ion volume, the eluting compounds came in direct contact with the chemical ionization plasma.

### *3.3.2. Analyte calibration and method detection limits*

Quantitation of the estrogens was done by calculating a simple linear regression equation for the peak area ratios of the target/deuterated estrogen pair and the estrogen concentrations followed by application of the equation to the sample data. Although the calibration standards were not evenly spaced, the $r^2$ values

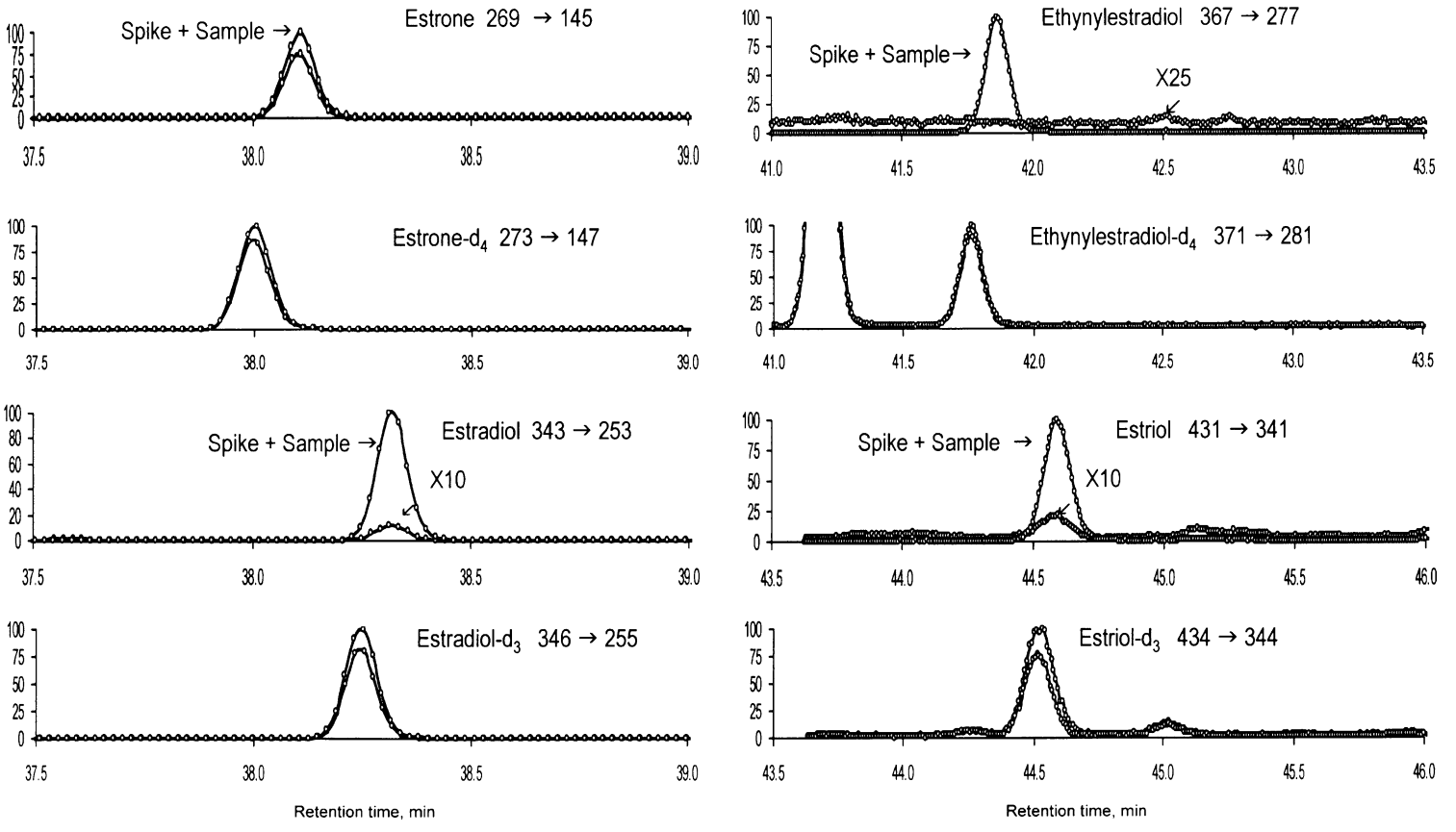


Fig. 6. GC–MS–MS mass chromatograms of the derivatized extract of lagoon sample FIN1 showing overlaps of the unspiked and spiked samples. The sample was spiked at 10,000 ng/l which corresponds to an extract concentration of 250 pg/μl.

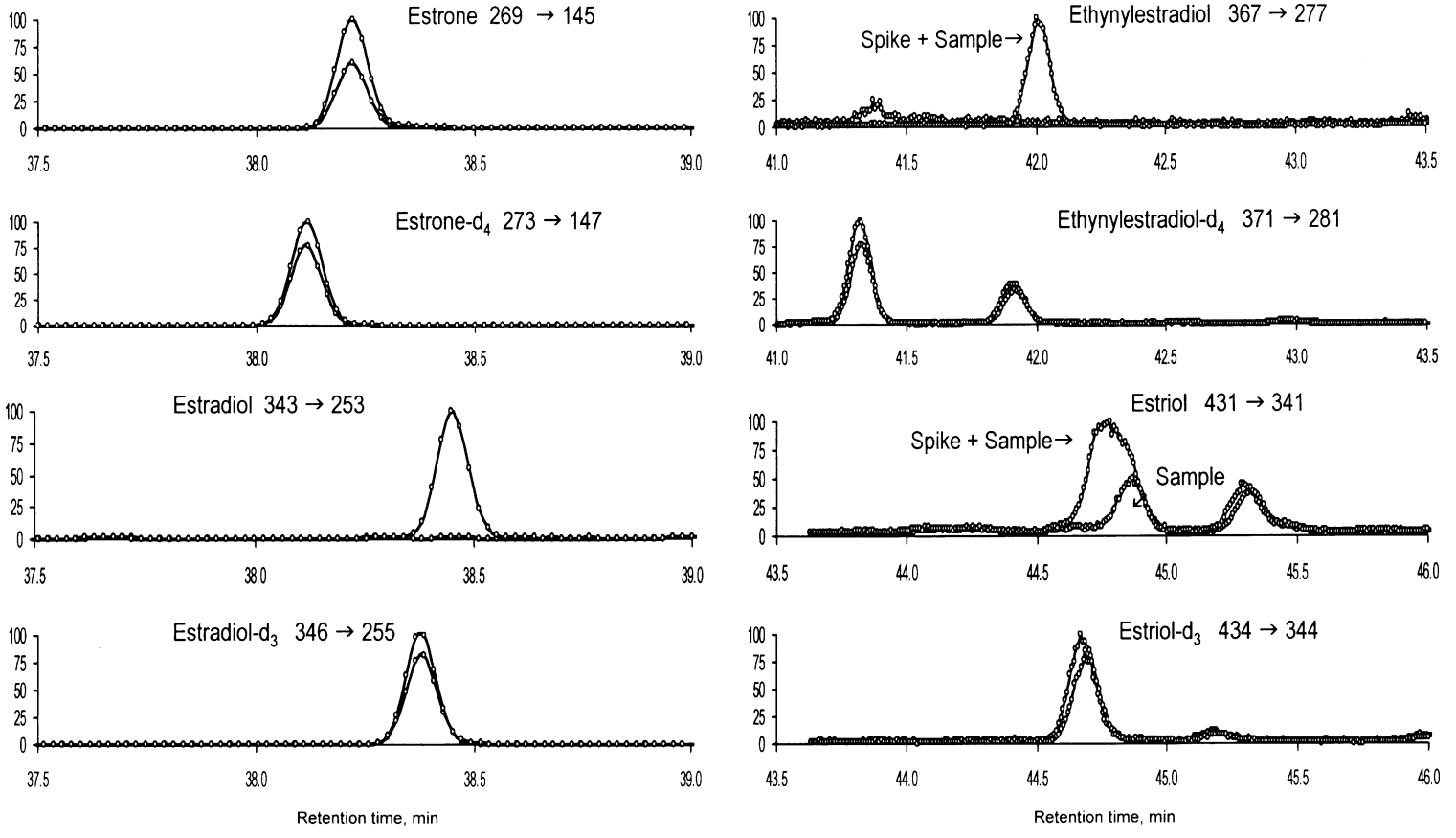


Fig. 7. GC–MS–MS mass chromatograms of the derivatized extract of lagoon sample SOW5 showing overlaps of the unspiked and spiked samples. The sample was spiked at 1000 ng/l which corresponds to an extract concentration of 25 pg/μl.

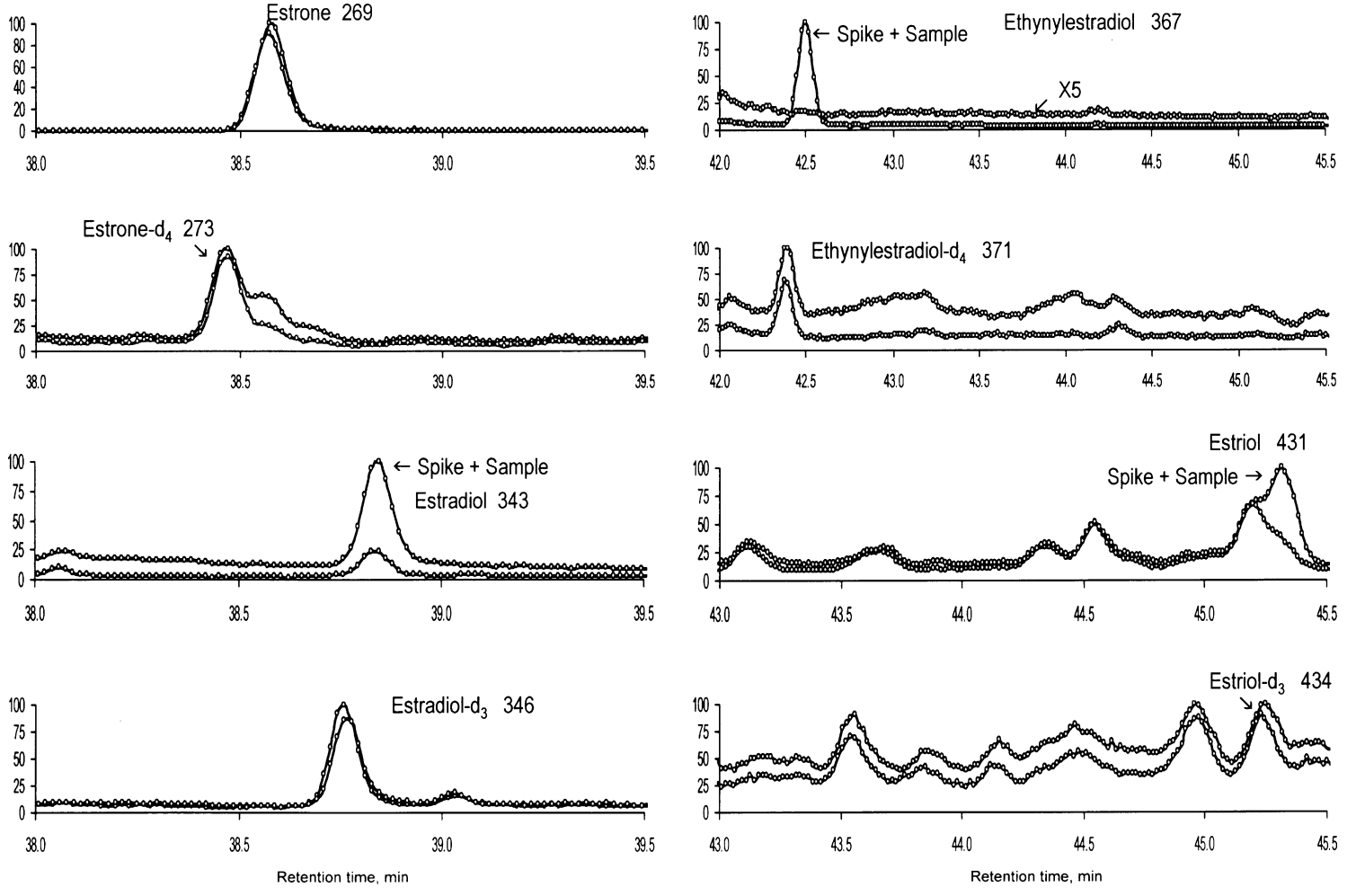


Fig. 8. GC–MS mass chromatograms of the derivatized extract of lagoon sample FIN1 showing overlaps of the unspiked and spiked samples. The sample was spiked at 10,000 ng/l which corresponds to an extract concentration of 250 pg/μl.

of the regressions for calibration curves prepared for 10 sample queues were high, and therefore data transformation or weighted regressions were not deemed necessary. The average $r^2$ value for forty calibration curves of the estrogens was 0.999 (S.D. = 0.003).

The MDL for the four estrogens in ground water was determined according to the standard procedure [32] by spiking 500 ml of sample from the CAFO sites with estrogens at 2.0 ng/l. Samples were chosen which did not contain measurable estrogens. The final extract concentration was 1.0 pg/μl. It was found that the MDL depends on the overall sensitivity of the instrument. When the ion volume is clean, MDLs ranging from 0.2 to 0.6 ng/l can be expected. When the ion volume became contaminated, the MDL increased to about 0.8 ng/l. Although the MDL was affected by the decrease in sensitivity, the effect on quantitation was minimal as long as the concentration of the sample was above the MDL. The responses for the target compound and its IDS were affected equally even though the instrument sensitivity changed. MDLs could not be determined for the lagoon sample matrix due the high concentrations of estrogens found in these samples.

#### 3.3.3. Relative retention time

The relative retention time (RRT) for each target estrogen with respect to its corresponding IDS was calculated for each standard and sample when a target estrogen was found in the expected chromatographic window. The RRT was plotted against the sample sequence number. The average and standard deviation of the RRT for calibration standards was calculated and control chart limits were graphed at the average RRT $\pm$ $3\sigma$. The control charts for estriol and ethynylestradiol showed that in four of the lagoon samples, the RRTs were outside the control chart limits (data not shown). When the chromatograms of unspiked and spiked samples were overlaid so that the isotope standards coincided, it was found that the spiked estrogen did not overlap the target peak. The retention time for these peaks varied by 3–6 s from the expected retention times. Monitoring the RRT of expected target peaks was found to improve the data quality by indicating some false positive identifications.

#### 3.3.4. Sample spike recovery

Spike recoveries of the target estrogens were determined in water blanks, ground water, and lagoon samples. For water blanks or ground water samples, 500 ml of sample was spiked at 2 ng/l with the target estrogens. The average recovery for the four estrogens in sixteen matrix spike recovery determinations was 103% (S.D. 14%). In five formaldehyde-treated lagoon samples spiked at 25, 500, 1000 and 10,000 ng/l, the average matrix spike recovery for the four estrogens was 103% (S.D. 15%). Table 3 shows the recovery of spiked estrogens from selected lagoon samples and the effect of formaldehyde addition. When formaldehyde was not added, the peak areas of the IDS, estradiol-$d_3$, in the spiked and unspiked samples decreased by 30% in NUR1, 95% in SOW5 and 99.7% in FIN1. For these three samples, the estrone spike recoveries were 146, 235, and 292%, respectively. This indicated that spiked estradiol was being converted to estrone prior to or during the SPE and that formaldehyde prevented this conversion. Oxidation of estradiol to estrone occurs naturally [3], and it is thought that either residual microbial activity, not removed by centrifugation and filtering, or some chemical oxidative process occurring on the surface of the SPE medium was responsible.

### 3.4. Analysis of field samples

#### 3.4.1. Estrogen concentrations in lagoon and ground water samples

Field data from the three swine facilities indicate that the primary swine lagoons contained fairly high levels of each of the three natural estrogens (Table 4). As expected, the synthetic ethynylestradiol was not detected in any of the lagoon samples. For the farrowing sow operation, estrogen concentrations in the digesters and primary lagoons ranged from 9600 to 24,900 ng/l for estrone, 5000 to 10,400 ng/l for estriol, and 2200 to 3000 ng/l for estradiol. Correlating information on estrogen distribution and concentration in swine waste is scarce, but these data are consistent with other results indicating that estrone is the primary natural estrogen in swine blood and urine, with estrone levels ranging from 1800 to 20,500 ng/l in female swine urine [33]. Lagoons at the farrowing sow facility were generally used sequentially (SOW1 to SOW5), although on occasion a primary digester was paired with one or the other primary lagoons (e.g. SOW1 to SOW3, SOW1 to SOW4, etc.). Hence, concentrations of the estrogens would not be expected to

Table 3
Recovery of estrogens spiked at 25, 1000 and 10,000 ng/l in swine lagoon samples and the effect of the addition of 1% formaldehyde on recovery

| Sample name | Concentration (ng/l) | | | |
|---|---|---|---|---|
| | Estrone | Estradiol | Eestradiol | Estriol |
| NUR1 | 464 | 35[a] | 0 | 185 |
| NUR1 + 1000 ng/l spike | 1930 | 474[a] | 814 | 1250 |
| Spike recovery (%) | 146 | 44[a] | 81 | 107 |
| NUR1 (1% form.) | 392 | 48 | 0 | 208 |
| NUR1 (1% form.) + 1000 ng/l spike | 1200 | 918 | 951 | 1340 |
| Spike recovery (%) | 82 | 87 | 95 | 113 |
| SOW5 | 80 | 190[a] | 0 | 6 |
| SOW5 + 25 ng/l spike | 139 | 16[a] | 25 | 30 |
| Spike recovery (%) | 235 | −698[a] | 98 | 97 |
| SOW5 (1% form.) | 81 | 0 | 0 | 15 |
| SOW5 (1% form.) + 25 ng/l spike | 106 | 25 | 29 | 38 |
| Spike recovery (%) | 102 | 100 | 114 | 92 |
| FIN1 | 75300 | 6350[a] | 0 | 214 |
| FIN1 + 10,000 ng/l spike | 105000 | 9990[a] | 9585 | 9460 |
| Spike recovery (%) | 292 | 36[a] | 96 | 92 |
| FIN1 (1% form.) | 74700 | 125 | 0 | 302 |
| FIN1 (1% form.) + 10,000 ng/l spike | 87500 | 8730 | 12200 | 10700 |
| Spike recovery (%) | 128 | 86 | 122 | 104 |

1% form. indicates the addition of 2.7 ml of 37% formaldehyde/100 ml of lagoon sample.

[a] Indicates that the peak area of the isotope dilution standard in these samples decreased by 20–300 times. Therefore, accurate quantitation is not possible. Listed values are shown for illustrative purposes only.

Table 4
Concentrations of estrogens found in lagoon samples

| Sample name | Concentration (ng/l) | | | |
|---|---|---|---|---|
| | Estrone | Estradiol | Eestradiol | Estriol |
| NUR1 | 392 | 48 | ND[a] | 208 |
| NUR2 | 576 | 40 | ND | 186 |
| NUR3 | 530 | 50 | ND | 175 |
| NUR4 | 623 | 48 | ND | 220 |
| SOW1 | 16900 | 3000 | ND | 8070 |
| SOW2 | 24100 | 2170 | ND | 10900 |
| SOW2 lab dup | 25700 | 2200 | ND | 9940 |
| SOW3 | 19200 | 2400 | ND | 7800 |
| SOW4 | 9590 | 2250 | ND | 5032 |
| SOW5 | 28[b] | 19[b] | ND | ND |
| FIN1 | 74700 | 125 | ND | 302 |

[a] Indicates no peak was detected.

[b] Calculated concentration was less than lowest calibration standard for lagoon samples (40 ng/l).

show a consistent decline along the numbered treatment train. Still, there appeared to be a gradual decline in estrogen values between the primary digesters and primary lagoons, and a very sharp drop in estrogen levels in the secondary lagoon SOW5. For the nursery operation, estrone was again the dominant estrogen in the swine lagoons, but concentrations of all estrogens were much less, as might be expected for a nursery versus a farrowing sow operation (Table 4). Concentrations were fairly consistent from one nursery lagoon to the next, as would be expected since each lagoon serviced a similar set of barns. Surprisingly, the highest estrone level was found in the finisher facility lagoon, with estradiol and estriol levels intermediate as compared to the nursery and farrowing sow operations. The reason for this is unknown, but this facility was not operated under the same general corporation as was the others, and this lagoon contained much more suspended matter (data not shown). Other factors could have been involved, such as the

relative age of the waste at the specific sampling location. Regardless, measured estrogen levels generally agreed with what might be expected for these types of operations.

Ground water samples from the farrowing sow and nursery sites were also analyzed for estrogens. Ethynylestradiol was not detected in any of the ground water samples. Estradiol, estrone, and estriol were either not detected or, if indicated by mass spectra, were present at concentrations less than the MDL (data not shown). The only exception was for estrone at one well at the nursery site, which contained 4.5 ng/l estrone. Coincidentally, this shallow well was adjacent to a stock tank for watering cattle, and therefore this value could reflect actual local contamination of the ground water. Additional sampling over time would be required to ascertain whether this represents an episodic event or is symptomatic of a more pervasive problem. It should also be noted that these monitoring wells were installed for the purpose of detecting leaks from lagoons, and not for assessing ground water contamination from land application areas or stock grazing areas. Other site data do not indicate conclusively that any of the lagoons are leaking and contaminating ground water (data not shown). Hence, although the estimates of estrogen concentrations in these ground waters appear plausible, other ground water sample points could yield substantially different data.

### *3.4.2. Phase distribution of natural estrogens in lagoon samples*

In order to ascertain the total mass of estrogens in swine lagoon samples, the distribution of natural estrogens in two lagoon samples was determined by analyzing the aqueous phase, the undissolved phase, and the inner surface of the sample container. For this analysis, the sample was centrifuged and the particulate/sediment phase was separated from the aqueous sample as before. The sediment phase was then washed three times with water, dried and IDS were added. The sediment phase was ultrasonicated twice with 10 ml of methanol and twice with 5 ml of acetone, similar to the methods used by Ternes et al. [34] for the determination of estrogens from sludge and sediments. The combined extracts were blown down and total estrogens were determined. The estrogens adsorbed to the glass surface of the bottle were likewise extracted using methanol and acetone. IDS were added to the bottle before extraction. For each estrogen, most of the mass was in the aqueous phase, with mass percentages ranging from 63–71% for estrone to 97–98% for estriol (Table 5). As expected for estrogens of decreasing polarity, the distribution for estrone content in the sediment phase was higher than for estradiol and estriol. Only small percentages of estriol, the most polar estrogen, was found in the sediment phase. The distributions of estrone and estradiol found in the swine lagoon sample compared well with distributions reported by Bowman et al. [35], who determined the partition coefficients for estrone and estradiol between water and estuarine sediment. Based on a 5 l distilled water sample, 2.5 g of sediment and 2.55 μg of estrone and 2.65 μg of estradiol, they found that after 200 h the sediment contained about 19% of the estrone and 15% of the estradiol. Similarly, Fürhacker et al. [36] found most of the radiolabeled estradiol activity to remain in the eluate from spiked and filtered waste water samples, with negligible sorption onto glassware. It should be noted that lagoon samples were obtained from the center rather than the bottom of the lagoons, since this represents the matrix used in land application, which presents the greatest potential environmental impact on ground water. Because these lagoons are not mixed, settling occurs with time and therefore the ratio of aqueous phase to solid-phase is very high in these samples. If

Table 5
Distribution of natural estrogens in aqueous phase, sediment, and on the inner surface of the sample bottle for two swine lagoon samples

| | Estrogen distribution | | |
|---|---|---|---|
| | Estrone | Estradiol | Estriol |
| SOW2 (%) | | | |
| Water sample | 71 | 84 | 98 |
| Precipitate | 22 | 16 | 2 |
| Bottle | 7 | 0 | 0 |
| SOW4A (%) | | | |
| Water sample | 64 | 84 | 98 |
| Precipitate | 25 | 15 | 3 |
| Bottle | 5 | 0 | 0 |
| SOW4B (%) | | | |
| Water sample | 63 | 74 | 97 |
| Precipitate | 27 | 25 | 3 |
| Bottle | 10 | 0 | 0 |

these lagoons were mixed, as one might find with an activated sludge treatment plant, the amount of particulate in the sample would be substantially increased and hence more estrogen mass would be present.

### 3.5. Method comments and considerations

In the original method described by Nakamura et al. [20], the final extract contained unreacted derivatizing reagent, TMSI. Our experience showed that injection of extracts containing excess TMSI causes damage to the GC stationary phase as evidenced by increased peak tailing and broadening. This problem was eliminated by addition of water to the final hexane extract to hydrolyze excess TMSI. The peak area of the derivatized estrogens did not change when this step was included. The addition of 100 μl of water to the hexane extract also aided removal of imidazole, one of the hydrolysis products. The hexane extract was not dried before injection.

Initially, an extraction method using ENVI-CARB cartridges containing graphitized carbon black support material was used for SPE. The method [15] using this SPE support involved a basic ammonia methanol wash of the cartridge after sample extraction and elution of the estrogens with methylene chloride–methanol (60:40). It was found that the Oasis HLB solid-phase media yielded better spike recoveries. It was also observed that extracts of the same sample were always darker using the ENVI-CARB medium compared to the Oasis HLB.

During method development it became obvious that method blanks and sample dilutions should not be prepared using tap water. When tap water, typically containing about 0.1 mg/l residual chlorine, was spiked with estrogens at 10 ng/l, spiked estrogens disappeared. Addition of sodium thiosulfate to the tap water before spiking with estrogens resulted in acceptable recoveries.

In order to determine causes for poor spike recoveries of estradiol in lagoon samples, several preservatives were tried. In all the lagoon samples tested, estradiol recoveries were always low with concurrent increases in estrone recoveries. When 1% trisodium phosphate was tried, a precipitate formed in those lagoon samples exhibiting high alkalinity. Evidence suggests that the estrone was absorbed to the precipitate and was lost when the sample was centrifuged. The use of phosphoric acid at pH $<2$ also caused precipitation. When 1% formaldehyde was added to lagoon samples, precipitation did not occur and excellent spike recoveries were achieved. However, when highly alkaline lagoon samples were treated with formaldehyde (pH $=$ 2.8–4), foaming of the sample occurred due to carbon dioxide generation. Moderate pressure in the sample bottle lead to spewing of the sample if the bottle was shaken before the cap was removed.

Initially, an LC–ESI-MS–MS method [4] was tried to determine estrogens in the swine lagoon samples, but ion suppression due to the extract matrix occurred as the estrogens eluted into the electrospray source. Instead of adding additional sample clean up steps such as silica gel or HPLC to the LC–MS–MS method, the GC–MS–MS approach using derivatization and NICI was selected to minimize sample preparation steps. Also, ELISA was considered as a screening tool to determine if estradiol was present in the ground water or swine lagoon sample. For ground water samples where quantitation limits of less than 1 ng/l was desired, SPE processing of the sample, including solvent blow down and extract reconstitution, was required to bring the estradiol extract concentration above the MDLs of ELISA (20 ng/l). Initially, the same SPE extract could have been used for both ELISA and GC–MS–MS, but with the addition of an IDS to the sample before extraction, a separate SPE extract became necessary for ELISA. For swine lagoon samples, an additional HPLC clean up with fraction collection was required for ELISA screening [26]. Due to the additional efforts required to prepare separate extracts for ELISA and GC–MS–MS, ELISA was not used to screen samples.

## 4. Conclusions

A sensitive method for the determination of estrogens in river water was improved to allow analysis of both ground water and swine lagoon samples. Changes in the method included the use of Oasis HLB solid-phase media to obtain better extract recoveries and cleaner sample extracts, and incorporation of SRM with MS–MS to minimize matrix coelution problems found with GC–MS analysis of swine lagoon extracts. The use of IDS for quantitation of each targeted

estrogen was shown to be important to account for decrease of mass spectral response which occurred when chemical ionization was used. When IDS were used, excellent spike recoveries resulted for both ground water and swine lagoon samples. Comparison of the RRTs for the IDS and the corresponding target estrogen aided in identifying some false positive identifications of targeted estrogens. Evidence was presented on the importance of using formaldehyde as a preservative in swine lagoon samples to prevent conversion of estradiol to estrone. The described method was used to determine the distribution of estrogens in the aqueous phase, the undissolved particulate/sediment phase and the container. As expected, the amount of estrogen adsorbed to the particulate/sediment decreased with the polarity of the estrogen. Finally, the described method was used to analyze estrogens in three different types of swine lagoons. Estrone was the predominate form of estrogens in each of the lagoon samples, and the synthetic ethynylestradiol was not detected in any of the lagoon samples.

## 5. Disclaimer

This work was funded wholly by the US Environmental Protection Agency (FPA) through EPA Contract No. 68-C-98-138 (Project Officer Georgia Sampson, US EPA National Risk Management Research Laboratory, Ground Water and Ecosystems Restoration Division, Ada, OK, USA), however, it has not been subjected to Agency review and does not necessarily reflect the views of the Agency, and no official endorsement should be inferred.

## Acknowledgements

The authors wish to thank Dr. Greg Jungclaus of Shaw Environmental for his efforts in editing the manuscript. The authors also wish to thank Mark White of the Ground Water and Ecosystems Restoration Division (EPA) for his assistance in collecting lagoon and ground water samples. All of the experimental work and initial manuscript preparation were done through EPA Contract No. 68-C-98-138 with ManTech Environmental Research Services, Inc.

## References

[1] L. Arcand-Hoy, A. Nimrod, W. Benson, Int. J. Toxicol. 17 (1998) 139.
[2] A. Belfroid, A. Van der Horst, A. Vethaak, A. Schafer, G. Rijs, J. Wegener, W. Cofino, Sci. Total Environ. 225 (1999) 101.
[3] T. Ternes, P. Kreckel, J. Mueller, Sci. Total Environ. 225 (1999) 91.
[4] C. Baronti, R. Curini, G. D'Ascenzo, A. Di Corcia, A. Gentili, R. Samperi, Environ. Sci. Technol. 34 (2000) 5059.
[5] M. Solé, M. Lopez de Alda, M. Castillo, C. Porte, K. Ladegaard-Pedersen, D. Barcelo, Environ. Sci. Technol. 34 (2000) 5076.
[6] E. Routledge, D. Sheahan, C. Desbrow, G.C. Brighty, M. Waldock, J.P. Sumpter, Environ. Sci. Technol. 32 (1998) 1550.
[7] G. Panter, R. Thompson, J. Sumpter. Environ. Sci. Technol. 34 (2000) 2756.
[8] I. Lange, A. Daxenberger, B. Schiffer, H. Witters, D. Ibarreta, H. Meyer, Anal. Chim. Acta 473 (2002) 27.
[9] US Environmental Protection Agency, Report EPA-821-R-99-002 EPA, Office of Water, Washington, DC, 1999.
[10] US Environmental Protection Agency, Fed. Reg. 66 (12 January 2001) 2959.
[11] O. Finlay-Moore, P. Hartel, M. Cabrera, J. Environ. Qual. 29 (2000) 1604.
[12] E. Peterson, R. Davis, H. Orndorff, J. Environ. Qual. 29 (2000) 826.
[13] M. Petrovic, E. Eljarrat, M. Lopez de Alda, D. Barcelo, J. Chromatogr. A 974 (2002) 23.
[14] M. Lopez de Alda, D. Barcelo, Fresenius J. Anal. Chem. 371 (2001) 437.
[15] A. Lagana, A. Bacaloni, G. Fago, A. Marino, Rapid Commun. Mass Spectrom. 14 (2000) 401.
[16] M. Avery, Rapid Commun. Mass Spectrom. 17 (2003) 197.
[17] M. van Hout, H. Niederlander, R. de Zeeuw, G. de Jong, Rapid Commun. Mass Spectrom. 17 (2003) 245.
[18] L. Sojo, G. Lum, P. Chee, Analyst 128 (2003) 51.
[19] G. Singh, A. Gutierrez, K. Xu, I. Blair, Anal. Chem. 72 (2000) 3007.
[20] S. Nakamura, T. Sian, S. Daishima, J. Chromatogr. A 919 (2001) 275.
[21] G. Biancotto, R. Angeletti, P. Traldi, M. Silvestri, M. Saccon, F. Guidugli, J. Mass Spectrom. 37 (2002) 1266.
[22] S. Cathum, H. Sabik, Chromarographia 53 (2001) S-394.
[23] X. Xiao, D. McCalley, J. McEvoy, J. Chromatogr. A 923 (2001) 195.
[24] H. Kuch, K. Ballschmiter, Environ. Sci. Technol. 35 (2001) 3201.
[25] O. Lerch, P. Zinn, J. Chromatogr. A 991 (2003) 77.
[26] C. Huang, D. Sedlak, Environ. Toxicol. Chem. 20 (2001) 133.
[27] R. Jeannot, H. Sabik, E. Sauvard, T. Dagnac, K. Dohrendorf, J. Chromatrogr. A 974 (2002) 143.
[28] Waters, Oasis Extraction Products: Environmental and Agrochemical Notebook Applications Notebook, Waters Corp.,

Estrogens in River Water at 5 ng/l (Endocrine Disruptors), 2002, p. 39 (http://www.waters.com/pdfs/loaeagan.pdf).
[29] M. Oehme, D. Stockl, H. Knoppel, Anal. Chem. 58 (1986) 554.
[30] G. Skarping, M. Dalene, P. Lind, J. Chromatogr. A 663 (1994) 199.
[31] M. Gross, Int. J. Mass Spectrom. Ion Process. 118–119 (1992) 137.
[32] Definition and Procedure for the Determination of the Method Detection Limit, Revision 1.11, U.S. Environmental Protection Agency, Code of Federal Regulations, 40 CFP Part 136, 1984, p. 554.
[33] C. Calvert, L. Smith, in: F. Coulston, F. Korte (Eds.), Environmental Quality and Safety, Georg Thieme Publishers, Stuttgart, Germany, 1976, p. 203.
[34] T. Ternes, H. Andersen, G. Gilberg, M. Bonerz, Anal. Chem. 74 (2002) 3498.
[35] J. Bowman, J. Zhou, J. Readman, Mar. Chem. 77 (2002) 263.
[36] M. Fürhacker, A. Breithofer, A. Jungbauer, Chemosphere 39 (1999) 1903.